Studies on Avian Hæmoprotozoa.

I. On certain Parasites of the Chaffinch (Fringilla cœlebs) and the Redpoll (Linota rufescens).[1]

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With Plates 27—31.

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1. Introductory.

My reason for taking up the study of Avian Hæmoprotozoa has been the desire to obtain, if possible, some definite enlightenment on the important question of their life-cycle. The far-reaching conclusions bearing upon this subject, to

[1] This research was carried out as Mackinnon Student of the Royal Society during the year 1907–1908. The publication of the results has been delayed for several months owing to a long stay at Rovigno in the endeavour to supplement this work by the study of the actual parasites described by Schaudinn in Athene noctua.

which the celebrated protozoologist, the late Fritz Schaudinn, was led as the result of his well-known researches (**27**) on certain parasites of the little owl (Athene noctua), have been largely discredited by many subsequent workers in this field. This is chiefly due to the suggestion, first put forward by the American workers, Novy and McNeal, that there is nothing in Schaudinn's description to show that the author took sufficient precaution against the liability of confusing the life-histories of what were really separate and independent parasites. Novy and McNeal, in their endeavour to confirm Schaudinn's views, investigated the trypanosomes of various birds (**14**), and also made a study of the flagellates occurring naturally in mosquitoes (**15**). As a result of their work they have maintained that Schaudinn was entirely wrong in regard to all his main conclusions. They consider, on the contrary, that the trypanosomes of birds are quite distinct from intra-cellular parasites (such as Halteridium), and further, that they do not undergo any part of their life-cycle in an insectan host, the flagellates occurring in the latter having no connection with the trypanosomes.

I chose avian forms on which to work for the following reasons: In the first place, a considerable amount of research has now been done on various trypanosomes parasitic in other vertebrates, e.g., fishes and mammals, which will be referred to in due course. Secondly, it is from a study of avian forms, if any, that one may reasonably expect to learn how far Schaudinn's views and statements were justified. As a matter of fact, at the present time the trypanosomes of birds are those about which the least is positively known, for Novy and McNeal's work, while it has undoubtedly re-opened the entire question, does not, on the other hand, contribute much to its definite settlement. In my opinion, many of the conclusions reached by these authors are equally open to criticism. They themselves have certainly not brought forward adequate or sufficient evidence to justify the negative views adopted by them.

Hosts Selected to Work upon.—It was my intention

to study first the parasites of the "little owl" itself. In spite of all my efforts, however, I could not obtain a supply of these birds here at home, so that I was obliged to turn my attention to other birds. Recent observations have shown that many kinds of birds harbour trypanosomes, and it is probable that their infection with these parasites is fairly widespread in nature (cf., for instance, the numerous American species which Novy and McNeal found to be infected). The only worker, to my knowledge, who has published any notes relating to the occurrence of avian trypanosomes here in England is Petrie (**21**), who observed the parasites in the blackbird, swallow, house-martin, song-thrush, chaffinch, and yellow-hammer; he failed to find them in the crow, sparrow, starling, or jackdaw.

Had it been my object to find trypanosomes in as many different birds as possible and to content myself with noting their presence, it would have sufficed to shoot various kinds of wild birds and examine them at once. This habit of describing and naming trypanosomes from one or two casual observations is unfortunately far too prevalent; it is one which adds little or nothing to our knowledge of the really essential points on which light is needed. For the purposes of my investigation I felt it was best to restrict myself to birds which could be obtained without much difficulty, and which were hardy and would live well in captivity. Hence, with a few exceptions at the commencement of the work, when I was endeavouring to "lay a course" as it were, I have used small native cage-birds, obtained from various dealers. Mentioning the exceptions first of all, in order to give a complete list, I began with some Java sparrows (Padda oryzivora), from which host a trypanosome, T. paddæ, has been described by Thiroux. But after spending some time fruitlessly in attempts to find this parasite, which was not present, and my limited supply of these birds giving out, I relinquished the search. In spite of great efforts to trap common birds, the only result was a

blackbird caught for me at Elstree, which died two days after receiving it. Neither in this, nor in another blarkbird, purchased, were any trypanosomes found. A barn-owl (Strix flammea), which was kindly given me by Dr. Dean, also proved negative.[1] I may add here that in one of the Paddas and in one blackbird Halteridia occurred, but sparingly; I thought it best, however, not to take up this aspect of the question at first, but to continue my search for hæmoflagellates and concentrate my attention on them in the first place, turning to the Hæmosporidia later, as should appear desirable.

The small birds, of which I have examined most, are closely allied members of the finch family (Fringillidæ, sub-fam. Fringillinæ), namely, greenfinches (Chloris chloris), chaffinches (Fringilla cœlebs), redpolls (Linota [Acanthis] rufescens), and linnets (L. [A.] cannabina). Trypanosomes were found only in the chaffinches and redpolls, so that for the greater part of the time I have occupied myself entirely with these. Unfortunately during the spring these birds also were very scarce and difficult to procure, and I was unable to replenish or augment my stock when I particularly wished to do so.

The occurrence of the parasites in these two hosts cannot be considered as at all rare. Out of twenty-two chaffinches examined, five were found to be naturally infected, sixteen birds were certainly uninfected, and one was doubtful. Neglecting this last,[2] the percentage works out at about 24. As regards the redpoles, trypanosomes occurred in three out of fifteen; eleven were uninfected, and one, again, was uncertain. This gives an approximate percentage of 21·5, which is not very different from that in the case of the chaffinches. As far as they go these proportions are reliable, because they are exhaustive—that is to say, the

[1] In the case of blackbirds this was not conclusive as to the absence of the parasites, for no cultures were made (cf. below, p. 658).

[2] Also in the case of the first chaffinch and redpoll no cultures were taken, as I had no tubes ready at the time.

negative side also can be relied upon, for reasons which are given below ; in this respect they differ from most previous tables and estimates of trypanosome-infections of birds. The figures suffice to show that, so far as occurrence is concerned, the birds with which I have worked do not bear out the dismal statistics given by many of the researchers (e. g. Ziemann, the Sergents, Dutton and Todd, etc.)

Intra-cellular Parasites in the Chaffinch. — In several of the chaffinches I noticed, when looking for trypanosomes, the presence of Halteridia ; except in one case, which I shall describe shortly, these were only scanty in number. I have also observed, in three cases, an interesting leucocytic parasite, which is quite different in appearance from the celebrated Leucocytozoon ziemanni of owls.

What is undoubtedly a similar parasite has been observed independently by Dr. Stevenson, of University College, in smears of the blood of a greenfinch, which he has kindly shown me for comparison.

2. Methods of Work ; Attempts at Transmission by Mosquitoes ; Technique.

Fresh blood was always taken, in the living bird, from a fairly large marginal vein of the wing, prominent where it crosses the arm on the inner side, immediately below the elbow-joint. A fine-pointed surgical needle of the triangular-bladed kind was used. It is essential that the point be sharp. Unless a clean prick is obtained, the blood does not exclude freely in a good drop, but suffuses beneath the skin, raising a swelling from which blood cannot be got satisfactorily. As a rule bleeding stops quickly. Should it give any trouble, a swab of cotton-wool, dipped in lysol, is applied to the wound and the wing closed up over it and held to the side of the body for a few minutes. The vein soon recovers from this little operation, and can be used again, if desired, in a couple of days or so.

Culture-tubes.—The use of culture-tubes has been of the

greatest service to me. I have developed and extended Novy and McNeal's method, making use of it not only on the dead bird, but also—what is much more difficult—on the living bird. In taking drops of blood for culture-tubes, the great desideratum is to get the region of the arm above-mentioned sterile if possible. The part is very well washed and gently rubbed first of all with cotton-wool soaked in lysol, particular attention being paid to the skin near the base of the feathers. The lysol must then be washed away with distilled water, which has been well boiled. Lastly, the water is absorbed as well as possible with more cotton-wool, which has been boiled along with the water, and from which the hot water is quickly pressed out. This is preferable to using loose wool and serves to take up most of the water, the warmth also helping in drying the part. It is most important to have the arm as dry as possible before pricking the vein, otherwise the blood spreads and runs over the surface. As it exudes, the blood is taken up by a sterilised Pasteur pipette, the drawn-out tube of which is long enough to pass into the expression-water of the culture-tube.

It is, of course, a much easier matter to get sterile inoculations from the bone-marrow, heart, etc., if the ordinary precautions are adopted.

If a culture-tube can be successfully inoculated with four or five drops of blood, I have found that in a few days (usually five to seven, sometimes fewer) one can generally say with confidence whether the bird was infected, according as the tube develops trypanosomes or not. Unfortunately, even with the greatest care, the inoculated tubes are sometimes badly contaminated before that time has elapsed. In such circumstances I never rely upon a negative indication, though I may add that now and again a positive result has been obtained where the medium had become contaminated. When I have been unable to get any cultures to develop in two or three sterile tubes taken from a bird, subsequent examination and culture of the bone-marrow after death have also proved negative. Hence I have regarded the

above as a reliable test of the presence of the trypanosomes in the living bird.

Culture Media.—The parasite from the chaffinch and redpoll lives and multiplies readily in a blood-agar medium, prepared either after Novy and McNeal's recipe, or according to Mathis' modification. At first I followed the American authors (see **14**, p. 265), but added only an equal volume of defibrinated rabbit's blood to the sterilised meat-agar, as I found this to be quite sufficient. Tubes so prepared always have an ample quantity of expression-liquid, in which the parasites thrive at any temperature from 20° to 25° C. A temperature of 28° to 30° C. was found to be too high, if it was desired to keep the tube for any length of time, as the trypanosomes soon die off, owing to their too rapid multiplication and exhaustion of the nutrient material. At the lower temperature the tube is all right for about twelve or fourteen days, and some of the trypanosomes will remain alive longer if a little salt-citrate solution is added to replenish the medium. If it is desired to keep the culture going for some time, however, it is necessary to make a sub-culture, after ten or twelve days, by transferring a drop of the medium containing the parasites to a fresh tube. By this means I have kept a continuous series of cultural forms, both from the chaffinch and from the redpoll, thriving and multiplying for six and a half weeks, the one having been transferred (sub-cultured) four times, the other, I think, only thrice. Had it not been for the accident of the temperature of the incubator rising to nearly 30° C. for two or three days, whereby the trypanosomes were all killed off, the cultures could apparently have been kept for as long as I wished.

The great drawback to this method is that, where, as in my case, a large number of the tubes are used, too much time and labour are involved in obtaining sufficient rabbit's blood. Mathis' modification (**10**), which I have now followed for some time, avoids this difficulty. In this method, ox-blood, which can be readily got from a slaughter-house, is used instead. A quantity is allowed to fall direct into a sterilised

receptacle, and at once defibrinated. As before, equal volumes of blood and agar are mixed. The tubes, when prepared, must be sterilised by the fractional method at a temperature of about 100° C. (under rather than over), for an hour or so on two successive days. This is necessary to ensure sterility.

Owing to this process, however, tubes prepared thus are often deficient in expression-liquid; to remedy this 1 or 2 c.c. of boiling salt-citrate solution (·75 per cent. salt + 1 per cent. sodium-citrate), are added to each tube, which is then left for a day or two before being inoculated; the liquid absorbs nutrient material from the solidified part. The trypanosomes will not live in salt-citrate solutions alone. I have tried various combinations of salt, sodium-citrate, and (or) citric acid, similar to those used in cultivating the Leishman-Donovan bodies, but with no success. For the practical purpose of ascertaining whether a bird is infected or not I have found these tubes to be, as a rule, as serviceable as the others; but I do not think they suit the parasites quite so well. The culture does not start quite as easily, and multiplication is often somewhat slow at first. It is at least four or five days before the trypanosomes can be found at all readily in a small drop taken for examination, whereas in the case of the other tubes three or four days usually suffice. Again, after a week or nine days the parasites tend to become very granular and altered, and large agglomeration-clusters form sooner. In short, the trypanosomes do not live "healthily" so long in this kind of culture as in the other.

I may point out, with regard to the macroscopic appearance of infected tubes, that in the case of the parasites with which I have been working, there is normally nothing indicative of their presence to be seen. A culture (if free from bacteria) looks just like an uninoculated tube. Even when the parasites are very abundant, the expression-liquid remains clear and unaltered in colour. Not once have I found the parasites on the solid part of the medium. They never form visible colonies or masses there. The only

instances where anything unusual is to be noticed are in old, used-up tubes, in which the liquid is full of clumps of agglomerated parasites, and many are degenerating and dying. These masses tend to settle to the bottom of the liquid, and may be apparent as a small quantity of whitish-yellow scum.

Inoculation of Birds with Trypanosomes.—I endeavoured to produce an infection with trypanosomes in birds which I had found to be uninfected. So far, the only means at my disposal of doing this has been by inoculating; and most, certainly, of my attempts in this direction failed. In all about twenty-five inoculations were performed, and only in three cases was any positive result afterwards observed, which might be due to the inoculation. Many of the failures resulted from attempts to inoculate other (uninfected) birds with the trypanosome of the chaffinch and redpoll. Thus, a couple of linnets, one of them inoculated twice, proved negative. Also a barn-owl was tried with no more success. I was rather surprised, however, to find that a canary, which I thought would be very likely to prove susceptible, refused to become infected. It was inoculated three times, twice from cultures, and once from fresh (infective) blood, mixed with a little salt-citrate solution.

A few words in connection with the modus operandi. To begin with, I inoculated the birds intra-pleurally, as recommended by Novy and McNeal, but I lost two or three redpolls straightway as a result of the operation. It was very cold weather at the time, and this may have conduced to their collapse. Since then, I have always found it much more satisfactory to do the birds intra-peritoneally or intra-muscularly (in the pectoral muscles). None of the birds so inoculated suffered any ill-effects, even though, occasionally, they were done in both ways at once. The "dose" was generally four or five drops (from one eighth to one sixth of a cubic centimetre) of the liquid in the tube. This contained, of course, numbers of parasites.

With regard to the three cases in which the trypanosomes were observed subsequently, I may point out that I had made

sure, by means of good cultures, that all three birds had no trypanosomes in the blood prior to the inoculation, and therefore I considered them to be free from those parasites.[1] Hence these are in all probability instances of successful inoculation. One case was that of a chaffinch inoculated with the parasites from a redpoll; another was that of a redpoll inoculated with a culture from a chaffinch. With regard to the third case, that of a chaffinch inoculated with a culture from another chaffinch, I have been very uncertain, owing in part to the different course the infection took, whether the appearance of the trypanosomes in this instance was really due to the inoculation, or was connected with the presence in this bird of Halteridium. I now think this was also a case of successful inoculation, for reasons which are discussed below (see p. 678).

Attempts to Transmit the Parasites by Mosquitoes.—It was a great disappointment to me that all my efforts to get mosquitoes infected with the trypanosomes from the birds have been fruitless. Both from Schaudinn's description of the infection of Culex with the trypanosomes from the "little owl," as well as on account of the known rôle of this insect as alternate host of the Proteosoma (Hæmoproteus) of birds, I thought it most likely that mosquitoes would prove to be the transmissive agents of the parasites—at any rate, the trypanosomes—of the chaffinch and redpoll.

Unfortunately I was baffled in the very initial stage of all the experiments. I was never able to get the mosquitoes to bite the birds. I have tried at different seasons of the year, late spring, summer, and early autumn, and at periods when the temperature has been quite high for this country. Most of my attempts were made with females which were bred out from larvæ. None of them, however, showed the slightest inclination to bite. Nor would they feed on a guinea-pig, with which I tried them occasionally. They would only take

[1] I have worked throughout on the assumption that if trypanosomes are present, they will occur, if sparingly, in the general circulation.

such things as sugar-water, banana-juice, or mashed date. And if they were not provided with something of this kind they soon died off.

I also obtained several batches of "wild" mosquitoes (females), thinking these might at any rate bite. Indeed, Prof. Minchin, who sent me some from Norfolk, said they were biting the horses in the open fields at the time. But here again I had no better luck. In fact, the Culex seemed to starve instead of feeding on the bird. I have kept batches under observation without food,[1] and seen their bodies gradually become attenuated, until, although placed for a couple of nights consecutively with a bird, and without other food, by the fourth or fifth day (since they last took food) many of them would be dead. The mosquitoes were nearly always placed with the bird in the late afternoon, and left with it all night. Care was taken, of course, that they should be perfectly able to get to it and feed if they wished. Now and again, also, I held a tube containing a few hungry-looking insects to the bird's body for a little time, displacing the feathers so as to expose the skin; and similarly with the guinea-pig. I tried keeping the mosquitoes in a biological incubator at a temperature of about 25° C. (77°–78° F.), for a day or two before using them, but this did not make any difference. Even small pieces of organs containing blood from freshly killed rats remained untouched so far as I could see. In short, all my efforts to induce Culex to take blood were unavailing.

What is the probable explanation of this unwillingness experienced of the insects to bite? Such a total failure in this respect was quite unexpected. Taking into consideration the results in this connection—fortunately more successful—since gained at Rovigno, I think that there is probably more than one reason for the above negative results. In the first place, the question of temperature and moisture in the air is very important. I found this to be the case at Rovigno.

[1] But not without water, a small dishful of which was always kept in the cage.

Until the beginning of June I had the same difficulty there. As soon, however, as the regular summer weather set in—a moist, sweltering warmth—there was no difficulty in getting the Culex to bite (once, at any rate). It must be remembered that all the research done on Culex hitherto, in this connection, from which it is known both to transmit certain hæmatozoa and to harbour flagellates (which in many cases are most probably hæmoflagellates), has been done in countries where a much higher average summer temperature is experienced than in England. And I do not think that I succeeded in getting sufficiently favourable environmental conditions in my laboratory attempts in London.

There is another probably equally essential point, of which I was not aware at the time of my (the above) experiments. According to Mr. E. H. Ross, in a report on the prevention of fever on the Suez Canal (Cairo : National Printing Department, 1909),[1] the mosquitoes (females) apparently desire to suck blood only after having been fertilised. As it happened, in my early work I kept the bred-out females separate from the males, of which I took no account, thinking they were not required (as, of course, they do not take blood). Hence those females used were certainly not fertilised. As regards the caught "wild" ones, however, it is just as likely that they were fertilised as not, so that some of these ought to have bitten, had other conditions been suitable.[2]

Another Possible Insectan Host.—Owing to my lack of success in this essential preliminary, I was left in the dark as to whether Culex was the alternate host of the Hæmatozoa of the chaffinch or not. I may point out in passing that a study of the cultural forms of the Trypanosome which I have

[1] See 'Nature,' vol. lxxix, 1909.

[2] In working at Rovigno, where I was able to breed out the Culex in greater abundance, I left the two sexes together, for the sake of convenience in dealing with the insects. In this case many females were fertilised, for I frequently noticed the little "egg-rafts" floating on the dishes of water in the cage. Probably those females which sucked blood had been fertilised.

obtained, and their comparison with various flagellates described in blood-sucking Invertebrates (cf. below), leaves no doubt whatever in my mind that these bird-trypanosomes have some alternate (doubtless insectan) host. But it is quite possible that, in the present instance, some other insect than Culex performs this rôle. I endeavoured to ascertain what other biting insect was likely to be concerned. Mr. Austen, of the British Museum, very kindly informed me of a small hippoboscid fly, of the genus Ornithomyia, which is an ectoparasite of various birds, especially to be found on nestlings.[1] Up to the present, however, I have been unable to obtain a supply of these insects.

It seems to me not at all unlikely that it is in this direction one must look for the alternate host. If this be the case, it is very probable that infection usually occurs while the birds are quite young, and before they leave the nest.

Early in the autumn I obtained a young redpoll, infected with trypanosomes, which could not have been more than two months old, if that, when bought; and as most of these little cage-birds are caught, I am told, as soon as they can look after themselves and before they finally leave the nest, this may very well be a case in point.[2] Unfortunately, owing to the hampering restrictions of wild birds' protection acts, etc., I could not get hold of any nests containing fledgelings for examination. Towards the end of the close season a bird-seller did procure a chaffinch nest for me, from which the young birds only flew away as he approached. This was well searched for insects, but contained none. I may add that I have never noticed any insects (fleas, lice, etc.) on my birds

[1] The Sergents have recently found (30) that a hippoboscid fly belonging to the genus Lynchia is most probably concerned in the transmission of the Halteridium of the pigeon. Lynchia, however, is not met with in Britain.

[2] An interesting observation noted by Danilewsky of trypanosomes being present in a young roller-bird only a week old also supports this view. The only alternative would be that of hereditary infection, which is extremely doubtful.

when examining or inoculating them; they always seemed to be free from anything of this kind.

Technique.—All my permanent preparations are in the form of smears made on slides. As a rule, the thinner the smear the better the result. In the case of very stout trypanosomes it happens occasionally that they are rather flattened out if the smear is too finely drawn; but in thick smears the parasites are often not well stained by the Romanowsky method, being too blue in appearance. As regards smears of the cultural forms, I experienced some difficulty at first, on account of the expression-liquid (the medium containing the parasites), of which the drop to be smeared consisted. This was quite clear in the fresh condition, but formed a sort of coagulum after fixation, which stained very readily. Hence the trypanosomes appeared to lie in a layer of substance, stained reddish, which was often somewhat dense immediately around them. This coagulated layer was much more noticeable in smears made from the first kind of tubes than it was when I used the second kind, to which salt-citrate solution was added. The only means of obviating the trouble was to make the film as thin as possible and to take care that no stain was deposited on the slide.

Fixation.—Most of my preparations have been fixed with osmic acid vapour; the few smears not so fixed were of little value as regards the trypanosomes. I make use of a 4 per cent. solution of osmic acid, placed in the bottom of a stain-tube, to which two or three drops of acetic acid are added. The slide to be fixed is placed in the tube as quickly as possible after the film has been drawn. A fairly deep or thick glass ring in the liquid at the bottom of the tube prevents the slide itself from getting wet. Slides are left in contact with the vapour from twenty seconds to half a minute, the shorter time particularly in the case of a smear from a culture. After fixing, the slide is placed in absolute alcohol for fifteen to thirty minutes, according to convenience. If the smear is to be stained by the Romanowsky method, it is not advisable to leave the slide in absolute alcohol for much

longer than half an hour; I have always found a longer period to be detrimental to the staining. I found this method of fixation to be the best for giving a correct idea of the size and general appearance and morphology of the parasites, whether trypanosomes or intra-cellular forms; and, for the sake of uniformity, all my figures are of individuals so fixed, so that one may be compared at once with another, without any ulterior considerations having to be taken into account.

Staining.—Nearly all my preparations are stained by some variety of the Romanowsky method. I have made use of two stains (or stain mixtures): one of them is the ordinary Giemsa solution, the other is a combination which I have found particularly good for cultural forms. The Giemsa solution was always used in the customary proportion of one drop of the stain to 1 c.c. of water. The length of time for which slides were allowed to stain varied in different cases. The period required to give the best results varies considerably at times, even when the smears have been fixed, so far as can be told, in exactly the same manner. For one thing, the temperature made considerable difference. I used the stain at the laboratory temperature, and whereas in the winter and spring forms in the blood required to be stained for twelve to eighteen hours to be successful, in the summer they would be excellently stained in three or four hours.

Cultural forms stain much quicker than the parasites in the blood, and need only about fifteen to twenty minutes in the stain; but the Giemsa solution was found to be not nearly so suitable for smears of cultural forms as the other method which I adopted; by this latter method the parasites themselves are more sharply stained, while the coagulated layer, which is often unpleasantly prominent as a reddish ground-substance, after Giesma, hardly stains at all.

In my particular method three solutions are made use of, as follows:

(1) A 1 per cent. solution of azure I, in equal parts of glycerine and methyl-alcohol.

(2) A 1 per cent. aqueous solution of methylene-blue (Höchst—an essential point), to which 5 per cent. of pure sodium-carbonate is added. This solution is kept warm at a temperature of 40° to 45° C. for a couple of days or so, when it is made up, after which it is ready for use.

(3) A 2 per cent. solution of eosin (also Höchst).

In using the stain, I have found that a mixture made up in the following proportions gives very good results[1] : four drops of each of the three solutions are added to 10 c.c. of distilled water. The different liquids are poured from small drop-bottles of equal size, the drop-bottles being the same as are generally used for Giesma. (The drops themselves of the different liquids are not, it may be noted, of the same size.)

By this method cultural forms are excellently stained in six to eight minutes ; and if any stain is deposited in the ground-substance it comes away readily with orange-tannin afterwards. In fact, on a good smear of cultural forms thus stained, it is often scarcely apparent macroscopically that there is anything at all on the slide. For staining trypanosomes in the blood, only forty to fifty minutes is required.

In all cases, whichever method of staining was used, the slide was well rinsed with tap-water after staining, and then a few drops of orange-tannin were poured on the slide for half a minute or so, to remove the excess of stain. If, after further washing with water, the parasites still appeared to be over-stained, either more orange-tannin or else acetone was added. The latter must be used extremely cautiously and quickly rinsed off, for though at first it only extracts the blue, it soon begins to take out the red from the flagellum. Eventually the slide was washed with distilled water and allowed to dry.

I have since regretted that, owing to the great scarcity

[1] These proportions can be varied, of course, as is found most suitable, in other cases. I may mention that I experimented some time using either (1) or (2) alone in combination with (3), in various proportions, but I never obtained anything like the good results that I did after using both (1) and (2) together.

of the trypanosomes in the blood, I was not able to make use of the iron-hæmatoxylin method of staining. For there is one distinct drawback to the Romanowsky method and its variations. While it may be regarded as giving, after fixation with osmic, a perfectly reliable presentation of the form and general structure of the body, it is now quite clear from the most recent research (see, for example, Minchin [**12**] and Minchin and Woodcock [**13**]) that the nuclear structure and details cannot be interpreted correctly by the aid of stains of this kind alone. This is owing to the invariable tendency of Romanowsky stains to deposit the red colour in excess around certain organellæ, especially small granules, which are thus overloaded with stain and artificially enlarged to many times their real size, often with the result that other cytological features are quite obscured.

Nevertheless, this characteristic behaviour of the Romanowsky stains being now proved and recognised, due allowance can be made therefor, and hence one is not likely to be seriously misled in the case of a study such as is here described, which deals chiefly with the comparative morphology and behaviour of different types of form. Further, it may be pointed out that results obtained by the use of the same methods throughout may be compared with confidence.

3. The Parasites in Relation to Their Hosts.

Numerical Scantiness of the Trypanosomes.—As a rule, the trypanosomes are extremely scarce in the peripheral circulation of an infected host. This fact renders it often an excessively slow and wearisome process to get hold of the parasites at all in a living bird, and hampers any work upon them more than can be imagined until such research has been attempted. Unfortunately, there is all but unanimous agreement among observers upon this point,[1]

[1] The only exception of which I am aware is indicated by a statement of Vassal (**36**) in describing a trypanosome from an Annam pheasant.

which it would be tedious to cite in detail (cf. the remarks by the Sergents [29], Novy and McNeal [14], Laveran [6], Dutton and Todd [4], and others). I will only add that Petrie, in the note already referred to, states that he could not find the trypanosomes in the blood of any of the infected birds, but only saw them in the bone-marrow. With respect to this numerical scarcity, birds are certainly the most trying of all vertebrate hosts. There can be no doubt that, owing to this factor, an erroneous idea has often been obtained of the prevalence of trypanosome infections among birds. This has been well shown by Novy and McNeal, whose adoption of the culture method is of very great value in this connection. It will sufficiently illustrate this to give the statement of these authors that, in the case of forty-three various birds where microscopic examination had failed to reveal trypanosomes, nineteen, or 44 per cent., were proved by means of cultures to have been infected.

To give now my particular experiences. Out of five naturally infected chaffinches only in one were trypanosomes ever seen in freshly drawn peripheral blood; in this case, I once saw an individual in a cover-slip preparation. The same bird was examined at intervals during three months subsequently, but I never saw any living parasites again. That they were still present in the general circulation, however rare, was proved nevertheless on three occasions by means of cultures. Once, determined to find this elusive parasite if possible, I took a few drops of blood and made several smears, which were fixed and stained. In six good-sized films, which were minutely and thoroughly searched, representing a labour of several days, only one trypanosome was seen! It is important to note that these observations were made during the early spring, from January to April. In the case of the trypanosome parasitic in the redpoll I was

This writer was in the happy position of being able to say that the parasites were not infrequent in the peripheral circulation. An individual could be found in every two or three fields (of an oil-immersion lens).

not able to see it in the peripheral blood at all during the first five months of the year, although in two cases I knew by means of cultures that the birds were infected. During the early autumn, however, I was able to find it in smears from a very young bird, which had probably not been long infected. The number of parasites on a fair-sized film varied from six to ten in September, but only from four to eight in films made in October.

Principal Habitat.—In general, the trypanosomes are most numerous in the bone-marrow; this is certainly their principal habitat. Two or three parasites can usually be found in a fresh cover-slip preparation from one of the long bones of an infected bird. But even here, at times, considerable search is necessary,[1] since the parasites are apt to be hidden by clumps of leucocytes, erythroblasts, etc. However, there is generally no difficulty in finding the trypanosomes in a carefully made smear of a small, teased-up fragment of bone-marrow. Thus, when the chaffinch above alluded to was killed, some of the smears from the bone-marrow contained twenty trypanosomes or more.

Artificial Infection.—Only in a couple of instances up to the present have I had the pleasure of finding trypanosomes at all plentiful in the peripheral circulation. One of these cases, at any rate, was certainly the result of successful inoculation. This was a chaffinch which was infected with a culture of the form from the redpoll. Examined previously, no parasites had been found in this bird. On December 19th it was inoculated intra-peritoneally with a fifteen-day culture. On December 21st, twelve days later, examination of the blood showed at least five trypanosomes in two fresh cover-slip preparations, which were not exhaustively searched; and permanent smears made at the same time proved to contain quite a considerable number of parasites—twenty to twenty-five or more on a good-sized

[1] Certainly in one instance, where I failed to find any parasites in a careful search of the bone-marrow, the trypanosomes subsequently appeared in a culture taken from this organ.

film. On New Year's Day also, two parasites were found in a living preparation without much difficulty. When next examined, however, on January 10th, only one trypanosome was seen in two cover-slip preparations, which were thoroughly searched; this indicated a marked diminution in numbers. And in one permanent smear taken at the same time I could not find a trypanosome at all. This bird was not looked at again until the beginning of February, when no trypanosomes were seen in a living preparation. Nevertheless, the parasites were still present, for a tube inoculated subsequently developed a culture; evidently the parasites had by this time diminished in number to their customary scantiness. Unfortunately, this chaffinch accidentally escaped soon afterwards, flying away through an open window.

A Strong "Mixed" Infection.—I have left to the last a consideration of my most interesting case. On March 20th I inoculated a chaffinch with a seven-day culture of the chaffinch form. Three good (i.e. sterile) tubes had been inoculated from this bird previously, and had not developed any parasites. Hence I was practically certain that there were no trypanosomes present in this bird. Examination of the blood at intervals from March 26th until April 3rd, that is, until fourteen days had elapsed since inoculation, proved negative, no cover-slip preparations showing any parasites, so that I was very doubtful whether the inoculation had been successful. About three weeks afterwards the bird was again examined with a like result, but to make the matter certain, a tube (the first)[1] was then taken. To my surprise this developed a culture, the presence of the trypanosomes being thus proved, although I had never seen them in the fresh blood. I propose to leave aside, for the present, the question of whence these trypanosomes had come.

[1] I had not made a culture on the occasions of the earlier examinations, thinking that if the inoculation had been successful the parasites would have been readily observed in the circulation, as in the other instance described.

This bird was then left alone for some weeks,[1] until with the approach of summer I decided to look at it again and see if the oncoming season appeared to make any difference in the number or condition of the parasites. Examining a cover-slip preparation on the afternoon of June 16th I was surprised to see numerous microgametocytes of Halteridium. The stimulus of cooling was causing many of them to rupture the red blood-corpuscles, and rapidly form and liberate the active male gametes. I had never seen any Halteridia in the preparations or smears made previously from this chaffinch; if this parasite was present then it must have been extremely scarce in the peripheral circulation.

I was so occupied with watching this process of the liberation of the gametes and in endeavouring to see actual conjugation stages (unfortunately without success) that I did not search these fresh preparations for trypanosomes. In permanent smears made at the same time, however, trypanosomes occur, but they are not numerous (half a dozen or so on a slide).

Having this abundant Halteridium-material, and knowing the bird to be infected with trypanosomes also, I determined to examine it in the night-time to see if I could obtain any phases connecting these two types of parasite. Blood taken at 1.30 a.m. on June 18th showed the same condition as regards the Halteridia, and, in addition, Trypanosomes were easily found, three and four respectively being seen in two cover-slip preparations without any difficulty; and there were probably several more in each. The trypanosomes seen were manifestly much larger than the Halteridia, and I saw no indications of a rapid transformation of the Halteridia into trypanosomes, or vice-versa; indeed, the only Halteridia observed free in these living preparations were the adult gametocytes, male or female, behaving in the

[1] The bird was not made use of during this period because I had now given up making permanent preparations when a living drop failed to show the parasites. I had learnt that the probability was so much against my finding any trypanosomes in a reasonable time.

usual manner. Many smears of the blood were made, some at once, others after waiting a moment or two, and with or without the addition of a drop of salt-citrate solution.

The bird was again examined on the afternoon of June 22nd, when one trypanosome was seen in two cover slip preparations after some searching. Another night examination was made about 1 a.m. on June 30th. Compared with the previous night examination there appeared to be as many trypanosomes present, but the mature Halteridia did not seem to be quite so numerous as before. After a similar procedure I at length killed the chaffinch (about 2.30 a.m.) in order to obtain smears from the internal organs—heart, liver, spleen, bone-marrow, kidneys, etc. Most unfortunately, I omitted to make any preparations from the lungs—an oversight which I have since greatly regretted. I need only mention here that the trypanosomes were afterwards found to be comparatively few in number in preparations from the bone-marrow, while in smears from the liver, etc., they are very scarce. As regards the peripheral circulation, the parasites are certainly more numerous in these night-slides than they are in those taken (from the same situation) in the daytime (afternoon). Hence there would seem to be, to some extent, a wandering of the trypanosomes from the internal organs (probably chiefly from the bone-marrow, which is their principal "internal" habitat) into the peripheral circulation during the night-time.

Halteridium in Relation to the Corpuscles.—As already indicated, the Halteridial infection of this bird was a very strong one, and the parasites were very numerous at this time; in fact, in some smears, for instance, from the liver, they are almost abundant. The Halteridia are of all sizes, from minute forms up to fully grown adults. Nearly all the parasites are intra-cellular. Until recently the only cases in which I observed any forms free from the corpuscle[1]

[1] Of course, ripe sexual individuals, which have become rounded off and liberated themselves from the corpuscles, are not included in this statement; neither are distorted or irregular individuals, which have

—in spite of much searching—were four or five instances in which a special kind of individual, with peculiar features, was found free in the plasma. Having been led, however, as a result of my observations at Rovigno, to again examine very carefully certain of my preparations made at night, I have now found here and there a few individuals of small or intermediate size, and apparently of normal appearance, free in the blood. It is noteworthy that these free individuals have been seen only in smears from the peripheral blood, and not, for instance, in preparations from the liver, where the parasites are most numerous. Hence I do not think that the first impression I formed, namely, that the Halteridia do not leave the blood-corpuscle in the course of their growth, can be sustained.

Occurrence of the Leucocytozoon. — The new leucocytozoon which I have observed occurred in three chaffinches. In two it was very scanty, only one or two isolated individuals having been noticed, and they were small. In one bird, however, which happened to be that which was successfully inoculated with Trypanosomes from the redpoll (see above, p. 659), the Leucocytozoon is not at all infrequent. The parasites are nothing like so numerous as the Halteridia are in the case just described, but there are certainly as many or more Leucocytozoa than there are trypanosomes on any smear. On one film more than twenty-five have been marked, and the slide has not been exhaustively searched for all the minute forms. Unfortunately, I did not detect this parasite in living, cover-slip preparations. For one thing, I was examining the chaffinch in which it occurred for Trypanosomes, which can be readily seen; further, as this species does not produce the characteristic spindle-like appearance of the host-cell, as in the case of nearly all other Leucocytozoa so far described, there was nothing about the parasites to catch

obviously been accidentally set free from a ruptured corpuscle in making the preparation, such as are occasionally met with.

the eye. If I passed over one in my search I doubtless took it merely for a large leucocyte.

4. Description of Trypanosoma Fringillinarum, n. sp.

(a) As Found in the Birds.

The trypanosomes from the chaffinch (Fringilla cœlebs) and the redpoll (Linota rufescens) most probably belong to one and the same species. The trypanosome once noted, but not described, by Ziemann, in 1898, was most likely this form; and the same applies doubtless to Petrie's observations (21) in 1905. The occurrence of trypanosomes in the redpoll has not been known hitherto; this bird is a new avian host for the parasites. I regard the trypanosome from these two birds as a distinct and new species, for which I propose the name T. fringillinarum.

I discuss below the question of the specificity of different trypanosomes, with reference particularly to avian forms. I will merely give here the chief reasons which lead me to consider all the different types met with in the chaffinch and redpoll as belonging to one species. In the first place the ordinary, or definitive form of the parasite, the type, that is, which affords in the existing state of our knowledge the chief basis of morphological comparison in a systematic study of different Trypanosomes, appears to be essentially the same, as regards form and structure, both in the chaffinch and in the redpoll (cf. for instance figs. 4 and 31 of individuals from a naturally infected chaffinch with figs. 3 and 32 respectively of parasites from a naturally infected redpoll). Again, the forms which appeared in the blood of a chaffinch as the result of inoculation with a culture of the redpoll-parasite are also of a similar type (cf. figs. 1 and 28).[1] Secondly, although considerable polymorphism is shown, transition forms occur, which are intermediate between the more

[1] The fact that the inoculation of the parasites from the redpoll into the chaffinch was successful itself points to the specific identity of the two forms.

extreme types noticed and serve to connect them. Lastly, it may be added that the various cultural forms to which the parasite from the chaffinch gives rise are quite similar to, and cannot be distinguished from, those developed from the trypanosome of the redpoll.

The ordinary or definitive type of T. fringillinarum is elongated and slender in appearance (figs. 1–4, 27, and 28) The aflagellar end is long and finely tapering, at times being, indeed, extremely attenuated (fig. 27).[1] The free flagellum is usually comparatively short. The trypanosome possesses a well-developed undulating membrane, which has three or four folds or pleats, broad and deep. The average dimensions of a full-sized "adult" individual are as follows:

Total length, including flagellum	41 to 45 μ
Greatest width, including undulating membrane	$4\frac{1}{2}$ to 5 μ
Greatest width of undulating membrane	$1\frac{3}{4}$ μ
Length of tapering aflagellar portion of body, i. e. the distance from kinetonucleus to extremity	5 to 7 μ
Length of free flagellum	5 to 7 μ

The trophonucleus (nucleus) is situated near the middle of the body, often slightly in the aflagellar half. It lies generally somewhat nearer to the undulating membrane than to the opposite side. The nucleus is more frequently ovoid in shape, but it may be approximately round (figs. 1 and 29); in the former case it may measure as much as 3 μ by 2 μ, and in the latter case it may have a diameter of $2\frac{1}{2}$ μ; but these dimensions are not always attained.

The kinetonucleus appears as a relatively large body,

[1] This aflagellar prolongation is very delicate and liable to be broken off and lost in the preparation of the specimen; hence, now and again a parasite is seen which appears to have no "snout" at all, and where the body appears to be terminated by the kineto-nucleus; this is certainly an artificial condition, for it is characteristic of the fully grown ordinary individuals to have this long attenuated process at the aflagellar end.

ovoid or rather oblong, which occupies the entire width of the parasite at the point where it is situated. Its apparent size is about $1\frac{1}{4}$ to $1\frac{1}{2}$ μ by 1 μ. It is nearly always intensely stained after Romanowsky stains, and shows no structural details.

The flagellum, at its proximal end, nearly always stops short of the kinetonucleus; only very exceptionally does it appear to come into contact with the latter organella. In this connection it may be emphasised that my specimens are all from films properly fixed with osmic-acid vapour—none from air-dried smears. Moreover, at the point where the flagellum terminates, a definite granule, staining rather more deeply, can sometimes be made out quite clearly (figs. 4, 28). Unfortunately in many cases the root portion of the flagellum, which is probably intra-cytoplasmic, is not well stained, and in these the granule cannot be made out.

The cytoplasm stains pale blue, and is of fairly uniform structure, appearing in some instances finely alveolar. Occasionally a few small vacuoles or spaces are to be seen in the cytoplasm, but I have not observed anything that could be regarded as a definite, regularly occurring organella of that kind. In some of these forms the cytoplasm is free from granules; in others, however, granules which stain bright red, and are of varying size, occur in greater or less number (figs. 1, 3, and 27). These granules are most probably of a chromatoid nature, derived from the nucleus.

The structure of the undulating membrane shows an interesting feature. Running longitudinally in the broad folds or pleats, usually about the middle, is a prominent line, which stains blue—not red, like the flagellar border (figs. 1, 2, 4, 28–32). With a good light it is not difficult to make out that the part of the fold nearer to the body appears slightly denser than that on the outer side of this line, and stains faintly but distinctly blue, whereas the outer part is practically colourless. The explanation of this structure is that it represents a delicate intrusion of the

endoplasm, running part of the way into the pleat of fold, between the two (otherwise) closely apposed ectoplasmic layers which constitute the membrane. The longitudinal line about the middle of the fold is the edge or limit of this inner endoplasmic layer. Laveran, in his account of T. avium (6), calls attention to a "rib" or longitudinal striation in the membrane. This striation corresponds, in all probability, to the limit of an endoplasmic intrusion similar to that just described.

Apart from the undulating membrane, I have never seen indications of an ectoplasmic layer. The trypanosomes I have studied show no sign of a well-developed, red-staining "periplast," such as has been described by several workers in the case of T. lewisi, for example. As a matter of fact, I should not expect to see any such appearance here, since the ectoplasmic part of the folds of the membrane is itself generally quite colourless, as already mentioned, and at most shows in one or two instances the faintest possible tinge of pink colour, which would be quite lost against the stronger blue of the body. Nevertheless, there is no reason to doubt that the parasites have a delicate ectoplasmic sheath, investing the body generally.

I will leave until later the consideration of the minute structure of the trophonucleus.

The above type of the parasite is the form which I have found in the blood of the host—at any rate, in the chaffinches—during the winter and early spring months, when the numerical factor is low, the infection being, as it were, persistent, but in a quiescent and somewhat scanty condition.

Young individuals, not yet full-grown, which belong to this ordinary definitive type, can be readily recognised. They are, of course, somewhat smaller, but their form and general appearance agrees in most respects with that of the adult parasites. The chief point of difference is that the "snout" is usually not so elongated and drawn-out; it is more conical, but still sharply pointed (figs. 31–33). This aflagellar part of the body attains the extreme degree of

attenuation only in the fully grown forms. An intermediate condition is seen in figs. 29 and 30. It will be noticed that there is often considerable variation in the size of the nuclei in these young or intermediate-sized individuals (cf. figs. 29–33), even where the parasites appear very similar in size and form. This feature is met with also in other series of forms to be described (see below, p. 672). I do not think much stress need be laid on apparent differences in size of these organellæ in comparing parasites otherwise similar.

Unfortunately, as already mentioned, I could not obtain any stained specimens of the parasite in the blood of the redpoll during that period, owing to its scarcity, although I had obtained cultures on two or three occasions. It was early autumn before I could obtain series of permanent preparations showing the trypanosomes in this bird; and in these smears, parasites which belong to the type above described are relatively scarce and outnumbered by another type. I have not found in this host at this period any ordinary forms which have attained quite the dimensions of the fully grown individuals occurring in the chaffinch in the early part of the year. The individuals observed, however, correspond closely to the slightly smaller forms of the parasite, which have been described above (cf., for instance, figs. 33 with fig. 4, and, again, fig. 32 with fig. 31). Hence I have little doubt that they represent that phase of the same species, bearing in mind also the other considerations stated already. It is probable that if I could have obtained examples of the trypanosome in the blood of the redpoll in the early part of the year I should have found "adult" definitive forms similar to those in the chaffinch.

The predominating form of the trypanosome in the blood of the redpoll in the autumn (September, and again in October), is a very large parasite. Some of the individuals of this new type are, in all respects, the largest trypanosomes I have observed in the birds, being not only as long as the longest ordinary individuals, but also much stouter. The individual drawn in fig. 37, for example, measures 48 μ in total length

and $6\frac{1}{2}$ μ in total breadth, while that in fig. 35 is 44 μ by $6\frac{3}{4}$ μ. Even the rather smaller forms of this kind (figs. 34, 36, and 38) are distinctly wider than the full-grown definitive parasites, their breadth varying from $5\frac{1}{2}$ to 6 μ. Hence, in general appearance these trypanosomes differ considerably from those of the first type.

The aflagellar end is prolonged for some distance (6 to 8 μ) beyond the kinetonucleus; it may be fairly wide and somewhat blunt (fig. 36), or slender and tapering (fig. 37), but it is never so finely drawn-out and attenuated as in the case of the definitive individuals. The free flagellum is usually short, only about 4 to $4\frac{3}{4}$ μ long. The undulating membrane is well developed, but the folds or pleats are not usually so sharply separated from each other as in the case of the other forms.

The cytoplasm of these massive forms stains blue, deeply and intensely.[1] In structure it is quite different from that of parasites belonging to the other type. As a whole it is much coarser in texture and more granular. In the majority of cases it does not appear to be of uniform character throughout the body (figs. 34 to 36). In the aflagellar third or so of the body it is loose and spongy, with large granules more or less uniformly distributed; but in the other two thirds or so, i.e. in the region from the trophonucleus to near the flagellar end, it is more compact, and the granules tend to be closely arranged in longitudinal rows, of which there are usually five or six. Thus the cytoplasm in this part of the body appears made up of narrow dark bands (composed of more prominent granules, packed together), with between them paler bands or zones of more finely granular (and hence less deeply staining) cytoplasm. The extent to which this serial arrangement of the larger granules is developed varies in different individuals. In some they extend through two

[1] There is no question of this difference being due merely to accidental variations in the staining; individuals representing the two types of form have been found on the same smear, and within a short distance of one another.

thirds or more of the length of the body, while in others they occupy only the middle portion (fig. 35). Now and again these bands appear very narrow, but in no case can they be considered as lines or striations; I do not think they have any connection with, or themselves indicate, actual myonemes. Dutton and Todd (4) have described what is probably a similar cytoplasmic differentiation in Trypanosoma mega and T. karyozeukton. They distinguish the loose, spongy aflagellar region as "spongioplasm," and the region of the longitudinal bands as "hyaloplasm." The chief difference in their cases is that the dark bands are very broad and very compact, showing less obviously their granular structure, while the alternating, less granular zones are very narrow and pale, and appear as clear stripes.

I have never seen any indications of division in any parasites belonging to either of the above types.

The next series of forms of Trypanosoma fringillinarum to be described consists, on the whole, of small parasites, some of which are extremely small. These forms have been found in two cases. The first instance of their occurrence noted was in the bone-marrow of a naturally infected chaffinch, which was killed about the middle of March. This bird had the usual scanty number of ordinary definitive trypanosomes in the general circulation, and these are also present in the bone-marrow, along with the parasites of small type. The other case was in the chaffinch which was found to have a mixed infection of Halteridia as well as trypanosomes towards the end of June (cf. p. 660). In this bird the trypanosomes were comparatively numerous in the blood; but no individuals of the ordinary large type have been found in any of the preparations, whether from the blood or organs. As I shall frequently have to distinguish between these two cases, it will be convenient, and will, I hope, render the description clearer, to refer to them as case A (the former, earlier case), and case B (the second, later case), respectively.

I will begin the account of this small type of form by

describing the parasites which occur in the later case (B). The smallest individuals have been found in the bone-marrow. The trypanosomes are distinctly less frequent in the bone-marrow than they are in the general circulation, and the individuals which do occur in this situation are nearly all small or minute in size. One of the smallest forms seen is drawn in fig. 40. Its total length is 15 μ, that of the free flagellum alone being 4 μ; hence the length of the body itself is 11 μ. The width is a trifle under $2\frac{1}{2}$ μ. It is only necessary to compare this parasite with some of those above described to realise the great difference in size which may be shown by different individuals of the same species of avian trypanosome. Another very small individual (fig. 5) has a total length of 18 μ, partly accounted for by the rather longer flagellum of 8 μ, and its greatest breadth is 3 μ.

On the other hand, the largest individuals belonging to this series of forms which I have observed are seen in figs. 44 and 45. The parasites are of only medium size; they do not really come in the category of large forms. The trypanosome of fig. 45 has a length of $33\frac{1}{2}$ μ, its flagellum alone is $8\frac{1}{2}$ μ, and the greatest breadth is $5\frac{1}{2}$ μ. The dimensions of the other individual are rather less. Between these two extremes of this type parasites of all intermediate sizes occur—forming, indeed, a regular gradation. This is illustrated by figs. 6, 42, and 43. The trypanosome in fig. 6, for instance, is 23 μ in total length, of which the flagellum is $6\frac{1}{2}$ μ, and has a width, including the undulating membrane, of $3\frac{3}{4}$ μ; again, the individual of fig. 43 is 27 μ long, the flagellum alone 6 μ, and the breadth $4\frac{1}{2}$ μ.

As will be noticed, there is a general similarity in form between all these parasites. The body is fusiform or spindle-shaped, and fairly wide in proportion to its length; it is quite distinct in appearance from the body of a definitive individual. The aflagellar end is drawn out and pointed, but it is not so elongated and attenuated as in the case of the definitive parasites described above. In the smallest indi-

viduals the undulating membrane is narrow and inconspicuous (figs. 40 and 5), but with the increase in size of the body it becomes wider and more prominent. The kinetonucleus may be relatively large, more particularly in the small individuals; in the parasite of fig. 5 it appears to be four-lobed, as if it were composed of four small masses. The free flagellum is fairly long, varying from $6\frac{1}{2}$ to $9\frac{1}{2}\,\mu$. A modification of this type occurs, but it is very uncommon in this series; certain parasites are relatively very wide, and have the aflagellar end very short and abruptly conical, which gives the trypanosome a stumpy appearance (fig. 41). The dimensions of this individual are: Total length, $18\frac{1}{2}\,\mu$; of the flagellum alone, about $3\frac{1}{2}\,\mu$; while the width is as much as $5\frac{1}{2}\,\mu$.

Comparing now the small forms present in the earlier case (case A), the parasites are quite numerous in the bone-marrow, and to this situation they appear restricted. They are of varying size, but I have not found individuals quite so minute as the smallest of those above mentioned. Parasites which are fairly small, nevertheless, are shown in figs. 46 and 47. The former is $25\,\mu$ in length and $3\frac{1}{4}\,\mu$ wide, the flagellum alone being as much as $9\frac{1}{2}\,\mu$; these two trypanosomes correspond fairly closely with that of fig. 6 from the other series, the chief difference being the longer flagellum. Here, again, it will be seen that there is considerable difference in the size of the kinetonucleus in the parasites compared. But on the same slide as the parasite of fig. 46, actually only two or three fields away, is another individual almost identical except that its kinetonucleus is nearly twice as large. Compare also figs. 44 and 45, and again, figs. 52 and 53.

Rather larger forms are seen in figs. 49–51. Most of the parasites in this earlier case, however, are comparable rather with the wide, stumpy form alluded to above, than with the fusiform individuals. Typical examples are seen in figs. 52–54. The parasite in fig. 54 has a total length of $27\,\mu$, the flagellum being $9\,\mu$, and its breadth is $5\frac{3}{4}$ to $6\,\mu$; the corresponding dimensions of the trypanosome in fig. 53 are

29 μ, 10 μ, and 6½ μ respectively. The flagellum of these trypanosomes is usually comparatively long (from 9 to 11 μ), being often longer than in the largest individuals of the fusiform kind. The kinetonucleus is always very near the aflagellar end, which is short and conical. The trophonucleus varies in shape; it may be more or less round, but it is often considerably elongated in a direction transverse to the longer axis (figs. 52–54).

It is noteworthy that in this earlier case no forms have been observed which correspond to the larger fusiform trypanosomes of the other series (case B). The parasites, which are no longer very small—which are becoming intermediate in size—such as the individual drawn in fig. 50, are obviously approaching in character the wide, stumpy forms, and differ appreciably from the intermediate-sized individuals of the fusiform variety in the features already indicated, namely, the broader body, the longer flagellum, and the abruptly terminating aflagellar part (cf. with figs. 43, 44, from the other case).

Many of the individuals in the above-described series of "small" parasites, including both fusiform and stumpy ones, show a cytological peculiarity which is at first somewhat puzzling. This feature is a row or chain of granules, which take up the red stain strongly, and which are very closely apposed to each other, giving the idea of a thick, beaded line (figs. 42–44, 47–50, and 54). This chain runs approximately parallel to the flagellar border of the undulating membrane, often following its curves closely, and it is frequently more deeply staining and prominent than the flagellar border itself. It begins near the origin of the flagellum, and always ceases with the limit of the body, at the opposite end, i.e. it never becomes free, as anything corresponding to a free flagellum. At first sight this line might be regarded as representing a new flagellum, formed either de novo or by a splitting of the old one, the parasites showing this appearance being therefore in the act of commencing division. After studying several of these individuals,

it is clear, I think, that this structure has really nothing to do with a flagellum. The line is usually most prominent in parasites which show numerous red-staining (probably chromatoid) granules in the cytoplasm; and, in suitable instances, it can be seen quite well that it is situated at the edge of the endoplasmic intrusion in the membrane (figs. 42, 47, 49). Further, when present, it can usually be traced right along the course of the membrane from end to end.

If we had to deal here with a case of division or formation of a new flagellum, individuals showing either an earlier or later phase in the process might be expected to occur, for this appearance is not at all infrequent; but I have not found any such. Again, in most cases, there is not the least indication of nuclear division. Lastly, in one of the exceptionally few instances where any indications of division are present, in addition to the kinetonucleus having divided into two, the true flagellum can be seen to be itself double for a short distance near its proximal end, probably as a result of splitting (fig. 54).[1] The granular chain is also present, and, as before, quite separate from the flagellum. Hence there is no reason for regarding this structure as in any way connected with a flagellum, much as it simulates one at times.

The small stumpy trypanosome in fig. 41 shows what is probably an early stage in the development of this line. Here there is a row of red-staining granules, quite separate, and not closely apposed to constitute a chain, which run parallel to the flagellar border, doubtless at the limit of the endoplasm. The granules are apparently quite similar to others which are seen in the general cytoplasm. I have no idea what is the explanation of this aggregation of chromatoid granules into a compact chain, lying in the position described. I have never seen it either in the ordinary definitive trypanosomes or in parasites of the other large type. I may add that I have observed the same feature in the case of a trypanosome from a blackbird (Turdus merula), at

[1] Cf. also the micro-photograph reproduced in fig. D.

Rovigno, the parasites which showed it being also of the same type of form.

There is still another variety of form to be mentioned, which occurs in case A (in the bone-marrow). This is a fairly small trypanosome (figs. 55 and 56), which is very narrow in proportion to its length. The aflagellar end is comparatively long and finely drawn out, and may approach the attenuated condition. The flagellum is fairly short, and the undulating membrane has well-developed folds. The dimensions of the individual in fig. 55 are: total length, 27 μ, breadth (including membrane), 3 μ, and length of flagellum $6\frac{1}{2}$ μ. The kinetonucleus is relatively large. These parasites strongly resemble in appearance young ordinary or definitive trypanosomes.

With regard to the multiplication of these small forms the only evidence I have been able to obtain is very slight.

I have observed three or four individuals (and not more) of the wide stumpy kind from case A, in which the kinetonucleus is in two parts (figs. 48 and 54); and in one solitary instance, just alluded to, the flagellum is partially doubled. In no case have I seen two trophonuclei. The condition in fig. 48 is the nearest approach to trophonuclear division that I have observed; this may represent commencing division because other organellæ of this parasite are dividing. The flagellum has not yet begun to divide, but as a prelude thereto, the centrosomic granule at its proximal end ("blepharoplast") is clearly double. So far as the fusiform series (of the other case) is concerned, I have observed absolutely no signs of division at any phase.

General Remarks.—The significance and relation to each other of all these manifold forms of the trypanosome is a somewhat difficult question. Where transitional forms or division phases occur they afford, of course, considerable help. Beginning with the small forms, the stumpy parasites of case A, in which indications of division can be found, probably give rise, as a result of that process, to small individuals like those in figs. 46 and 47, which grow into

somewhat larger individuals of the fusiform type (figs. 49 and 51). The stumpy trypanosomes themselves are best regarded, I think, merely as division-forms of young to medium-sized individuals of fusiform type. Hence, in this case, it may be said that the fusiform parasites present are of small to medium size and tend to multiply, by passing into the stumpy division form, rather than grow, at any rate at this period, into large trypanosomes. Next, with regard to the very thin, slender forms (e.g. figs. 55 and 56): when first seen they appeared in such sharp contrast to the prevailing stout type of parasite that I was somewhat disposed to think they represented male forms. As above mentioned, however, I am now more inclined to look upon them as young definitive parasites, which would grow into medium-sized ones, such as those in figs. 4 and 31, and so to full-grown adults, as in figs. 2, 28 (all from this series).

Turning again to case B (the later case), we find no ordinary forms present. Fusiform individuals of medium size are not uncommon, and between these and very small forms parasites of all intermediate sizes occur. There are very few stumpy forms, and none of those found show any actual signs of division.[1] Hence, the main condition here is undoubtedly a series of steadily growing fusiform individuals.

There remain two or three interesting questions in connection with the different type or phase of the infection occurring at different periods, in regard to which I can only put forward those surmises which seem to me the most probable. In the first place, comparing the condition found in a chaffinch (case B), in the summer, with that obtaining in a redpoll in the early autumn, where the parasites are mostly of the large massive type (e.g. figs. 34–36), I think it is most likely that the fusiform parasites of the former case (such as those of figs. 44, 45), would grow ultimately into individuals corresponding to those of the latter. The body-form is essentially similar in the two cases. The size of the

[1] It is possible, however, that the two or three small stumpy individuals seen in this case may be about to divide.

parasites found in the autumn is of course greater, but the difference is not relatively more than that between the larger and the smaller fusiform individuals in the summer. A difference which might appear of more importance is that in the character of the cytoplasm in the two cases. This can probably be explained, however, by supposing that the cytological features shown by the large massive individuals in the redpoll have become more developed and consequently more prominent, as a result of the increase in size. And, on the other hand, there is no evidence whatever that the fusiform parasites will pass directly into the characteristic ordinary type.

Assuming, then, this connection between these two sets of forms, how are we to explain the condition met with in the winter and early spring, when the only type of individual in the blood is the ordinary definitive form? The answer to this depends largely, I think, on what significance is to be assigned to the large massive forms just referred to. Are they to be considered as sexual individuals —of the female type? This is, of course, possible, but more than that cannot be said. And if this is the case, I certainly do not know which are the individuals of male sex; there do not appear to be any forms present at the same time which could be so regarded. On the other hand, I think it is at least quite as probable that the massive individuals have grown to this size prior to multiplication; they may later undergo some process of multiple fission or segmentation, occurring in one of the internal organs, and so give rise to the small forms. This supposition would fit in very well with the condition found, for instance, in case A (in the spring), where, as we have seen, small parasites are numerous in the bone-marrow, along with the ordinary forms, the latter being probably to some extent replenished from them. And here there are no signs of the large massive individuals. At all events, in view of Chagas' recent important work (2), showing that a new human trypanosome, Schizotrypanum cruzi, has a method of multiplication by multiple fission or

schizogony, I think it is not at all unlikely that naturally occurring trypanosomes—about whose life-cycle in the Vertebrate host very little is yet really known—may show some such schizogonic process more commonly than has hitherto been supposed.[1] In default of such a process in the present case, I have no idea how the small forms are developed, since they certainly do not appear to be derived from the adult ordinary individuals.

Another question is, What becomes of the ordinary, definitive forms of the trypanosome? As I have obtained many successful cultures from birds where this was the only type present in the blood, the natural inference would be that this form can be transmitted to the insectan host; but the same applies equally, it must be noted, to the fusiform parasites of case B, since I obtained cultures from them also. And I cannot be certain that both these types would develop naturally in the insect. Some of the ordinary forms, later on in the season, may pass into the large, massive type; this is not at all unlikely, if the latter is really a multiplicative form. The individual drawn, for instance, in fig. 39 may perhaps represent an intermediate stage in such a transition. Another possibility, of course, is that this definitive type disappears altogether in the summer, its place being taken by the fusiform type; the condition of the infection would then correspond with that of case B. I do not think this is likely. Case B most probably represented a recent infection (see below); in such the condition may quite likely differ from that found in an old established infection. Moreover, in the earlier case A (about the middle of March), parasites of the ordinary type are quite numerous, and do not look like disappearing; and further, in the autumn, in the redpoll, this type is also present.

It remains for me to say a few words with regard to the origin

[1] A most interesting piece of evidence bearing upon this point is supplied by Minchin (**12**), who mentions and figures the occurrence of a large individual of T. percæ, which is apparently in an encysted condition. Such a form might very well be about to undergo schizogony.

of the infection in this later case B, the chaffinch in which there was also an abundant halteridial infection. As I have stated in my note (**38**) on this interesting Halteridium, I was at the time inclined to think that the very small trypanosomes might have been developed directly from the Halteridia. Paying attention, for the moment, only to the trypanosome side of the question, in addition to the fact that in this case we have certainly to do, not with division, but with growth and increase in size from the minute forms up to comparatively large ones, there were other reasons which led me to take this view. This chaffinch, originally free from trypanosomes, was inoculated with cultural forms, but the subsequent course of events was very different from that in the case of the other successful inoculation described. In the latter case the parasites soon became comparatively numerous in the blood, whereas in the former they were not found at all at first, and only after some weeks were they shown to be actually present, by tubing (for further details, cf. p. 660). When at length they did become sufficiently numerous to be found without difficulty in stained preparations, they proved to be, as we have seen, quite different in form from the ordinary individuals developed in the other case. Hence, taking all things into consideration, I considered that the trypanosome infection was probably not due to the inoculation (which, in several cases, it must be remembered, did fail), but to the presence of Halteridium.

I admit now that I have changed my opinion about this case since writing my former note. In spite of the many features which seemed either to point strongly to this view, or at least to favour it, I think after all the trypanosome infection was not really connected with the Halteridial one, but was due to the inoculation (for further discussion of this subject, see under Halteridium). There remains the question, Why was the course of the infection so different in the two cases? Of course, in the one case where the parasites developed quickly, the inoculation was made with cultural forms which had come from a redpoll, while in the other they came from a

chaffinch; but I do not think this sufficiently explains the difference, because everything points to the species being the same in both birds. Since I have been able to study my cultural forms, I have come to the conclusion that the progress of the infection may have been so different, on account of a difference in the condition of the two cultures. The chaffinch-culture, from which resulted, we must suppose, the slowly developing infection, was one of six days' age, and certainly contained the characteristic trypaniform individuals to be subsequently described (cf. below, p. 690); for permanent preparations were made at the same time which showed this type. On the other hand, the redpoll culture used in the other (earlier) case was a fairly old original one of fifteen days; preparations were not made from this culture actually on the day when it was used for inoculating the bird, but in smears taken a couple of days before, none of these forms had been seen; the culture appeared quite healthy, and consisted almost entirely of the usual trypanomonad forms, to which, presumably, the infection must be ascribed.

It is an interesting question in which of these cases the course of the infection, so very different in the two, more nearly resembles that occurring naturally, i. e. by the inoculation of the right developmental forms from the insect. As will be seen on reference to one or two papers discussed below (p. 709), the remarkable trypaniform type alluded to is thought to be probably the true propagative form, which produces the infection of the vertebrate host. If this is so, it would seem to follow that the later case (case B), where the infection developed slowly, agrees most with the course of events in a natural infection.

(B) The Trypanosomes as Found in Cultures.

Before beginning an account of the cultural forms, one or two introductory remarks are necessary. When I commenced to make use of the cultural method, I did so solely because, from Novy and McNeal's work (14), it was evident

that it is of very great service in ascertaining whether a bird is infected with trypanosomes or not. I think now that I must have been unusually fortunate in my first experiences of the culture method.[1] I had no difficulty in getting the parasites to develop in my cultures, and, moreover, in a perfectly healthy manner. I soon had no trouble in distinguishing between what could be regarded as normal types, of regular occurrence, and what were abnormal, irregular forms. Hence, I admit that I modified my former attitude towards this method, and came to the conclusion that the cultural forms were probably, for themselves, well worth studying. I claim some excuse for my earlier opinion, since at that time this method had only begun to be adopted for trypanosomes, and in the early descriptions of cultural forms most of the figures depict what can only be described as altered appearances, which certainly belong to the category of abnormal phases. As a result of my own work, the view I now hold, and which I have expressed in my article in Lankester's 'Protozoa' (**39**), is that the cultural forms of trypanosomes may afford indications of value as to the developmental phases of the parasites occurring in the invertebrate host.

As I have already indicated, the chief cultural forms developed from the trypanosomes in the redpoll are quite similar to, and practically indistinguishable from, those to which the parasite from the chaffinch gives rise. I have had, however, a much greater number of successful cultures from the latter bird than from the former; hence I have found a greater variety of intermediate phases in my cultures from the chaffinch, and have had the good fortune, moreover, to observe one or two particular phases which I have not seen in cultures from the redpoll. This is doubtless due, however, merely to lack of sufficient material in the

[1] I may mention incidentally that I have since had a full measure of the trials and troubles which may attend the cultural method, for at Rovigno, in connection with the trypanosomes of the little owl, I had no success at all with it.

latter case, and I have no reason whatever to think that one set of cultural forms shows any intrinsic differences from the other, which would imply that the trypanosomes from the chaffinch and the redpoll, respectively, are distinct parasites.

The predominating type of the trypanosome in the cultures is a well-defined and characteristic form, which may be termed the trypanomonad form of the parasite, deriving this convenient general designation from one of the various alternative (synonymous) names (viz. Trypanomonas) given by Danilewsky to certain parasites described by him. This type is elongated and slender, the width usually varying but slightly in the middle of the body, and diminishing more or less gradually towards the aflagellar end. The essential diagnostic characters are: (1) The two nuclei are always close together, and situated either about the middle of the body, or else distinctly in the aflagellar half; and (2) the flagellum is attached for some distance to the side of the body, forming a distinct undulating membrane. The membrane may be at times fairly prominent, and possess a wavy edge, indicating a slight development of pleats or folds. The kinetonucleus is never near either end of the body. It is important to note that the flagellar end of the body is drawn out with the flagellum, as it were, and ultimately thins away, leaving the flagellum free. This condition is of very general occurrence, of course, among trypanosomes (as seen in the blood), and is the natural consequence of the presence of an undulating membrane. In respect of all the above features, therefore, the trypanomonad type differs essentially from a herpetomonad form.

Typical examples of the trypanomonad form, showing parasites of medium to large size, are seen in figs. 7, 8, 71–75, and figs. 13, 77–79, from preparations of cultures from the chaffinch and redpoll respectively. To give an idea of the size of these forms, three principal measurements may be taken: (A) length of body alone, (B) greatest width of body, and (C) length of free flagellum. These dimensions, in the case of some typical individuals, are as follows (in μ); fig. 72—(A)

21, (B) 3, (C) 9; fig. 73—(A) 25, (B) $2\frac{1}{2}$, (C) 10; fig. 7—(A) 25, (B) $2\frac{1}{2}$, (C) 29; fig. 75—(A) 26, (B) $3\frac{1}{4}$ (opposite nucleus); (C) 11; and again, fig. 79—(A) 21, (B) $2\frac{1}{2}$, (C) 15; fig. 77—(A) 23, (B) 3, (C) 19; fig. 88—(A) 26, (B) $3\frac{1}{2}$ (opposite nucleus), (C) 14. The measurements are given in a slightly different manner from that adopted in the case of the parasites when in the bird. In the cultural forms the length of the body by itself affords a better means of comparing the size of different individuals than the length of the body plus that of the flagellum. This is because of the great and apparently indiscriminate variation in the length of the flagellum, which cannot be said to bear any relation to that of the length of the body. This is well seen by contrasting figs. 80 and 81, from a redpoll culture, with figs. 84 and 83, respectively, from a chaffinch culture. This diversity is chiefly due to the manner of division, as will be explained shortly.

Smaller forms, very similar in appearance to some of the larger ones indicated, are seen in figs. 85 and 86; the former is 17 by $1\frac{3}{4}\,\mu$ and its flagellum $7\frac{1}{2}\,\mu$. The smallest parasites observed, however, belong to, or result from, a slightly modified variety of the above type. This is somewhat different in appearance (figs. 8, 97), but it really represents only another facies, as it were, of the same trypanomonad type, from which it is derived by the gradual drawing back of the nuclei well into the aflagellar half of the body, and by a somewhat modified manner of division which is then found (concurrently).

As the process of multiplication plays an important part in the development of these various forms, it may be as well to give a general morphological description of it here before proceeding farther. The mode of division by which the long, slender trypanomonad forms are produced is that of equal or subequal fission of the body. Sometimes the two daughter-flagella are practically equal (figs. 11, 96), but in the majority of cases one of the flagella is distinctly longer than the other (figs. 91–95). In all the instances I have noticed, the division of the cytoplasm begins at the flagellar

end. It generally happens that, as the split extends, the parasites tend to separate from one another, turning outwards, away from each other as it were (figs. 92, 93); eventually the two daughter-individnals come to lie in one line (which may be more or less curved), with the flagella,waving freely at opposite ends, the parasites only remaining connected by what is actually the still undivided aflagellar end (figs. 94, 95). The fact, therefore, that we may find either equal or sub-equal cytoplasmic division in which the daughter-flagella differ considerably in length, explains the great variation in this respect which is met with among the ordinary trypanomonad individuals.

In many cases the division of the two nuclear bodies does not take place in a direction quite transverse to the long axis of the body, but in an oblique direction, one pair of daughter-nuclei lying somewhat nearer to the aflagellar end than the other pair. In this manner are produced forms such as are seen in figs. 8, 97, and 100. These individuals in which the nuclei have progressed into the aflagellar part of the body have the undulating membrane very well developed; it may be said that the trypanomonad condition is here accentuated. In such forms of the parasite the mode of division is also distinct, being markedly unequal in character (figs. 98–100). The two resulting individuals are not of the same type (cf. figs. 12, 103, and 107). One, the larger parasite, is of the same type as the parent individual, and possesses from the first a conspicuous membrane, but the other, the smaller daughter-individual, is at first pear-shaped and stumpy, and has only a short, inconspicuous membrane. This mode of division presents a general resemblance, it will be noted, to one of the types of division characteristic of T. lewisi. Indeed, in the present case, the process might also be regarded as a "budding-off" of a daughter-individual from the parent. I have never observed, however, more than one bud formed, i.e. the process appears always to retain its character of binary fission and never to be of the multiple type. When set free, the smaller

daughter-individual elongates a little and becomes spindle-shaped instead of pyriform; the membrane also becomes more conspicuous. I have not seen any transitional phases between these fusiform individuals and the type represented by the parent form, and have therefore no indications as to whether they (the former) grow or otherwise pass into the accentuated trypanomonad type again. By successive multiplication according to this manner the size of the parasites become considerably reduced. In fig. 102 is seen a very small couple of the kind described. Examples of free parasites, of different sizes, representing accentuated trypanomonad daughter-individuals are given in figs. 105 and 108–110, 108 being from a redpoll culture, the others from a chaffinch one. The smallest form (fig. 110) is $10\frac{1}{2}\,\mu$ long, its flagellum is $13\,\mu$, and its breadth is $2\frac{1}{4}\,\mu$. The small fusiform parasite of fig. 111, representing a pyriform daughter-individual, is $9\,\mu$ long, its flagellum is $7\frac{1}{2}\,\mu$, and its width $2\frac{1}{2}\,\mu$.

The great majority of the parasites in thriving cultures belong to the above-described types. After a fresh culture-tube has been inoculated (from a bird) about five days, by which time the trypanosomes have generally multiplied sufficiently to ensure that there will be a fair number of parasites on a permanent smear—in other words, that an individual can be found without much searching—practically all the parasites present conform to the trypanomonad type. And up to the end of a week or so this type persists with great constancy, notwithstanding the rapid multiplication. The only variations that are numerically important are those already indicated, in the direction, that is, of an accentuated trypanomonad type and of a fusiform one. Further, if a sub-culture of these normal forms is made (preferably not later than the seventh or eighth day) the development of similar forms continues steadily in the sub-culture. Thus the parasites drawn in figs. 74, 109, are on a preparation from a second sub-culture, and the total interval that had elapsed since the blood was originally taken from the bird was twenty-six days, or over three weeks.

Certain other phases or developmental forms of the trypanosomes, however, have been encountered in cultures which were in a normal healthy condition, but these have been, as a rule, scanty in number, contrasting markedly with the abundance of the prevailing types. In cultures of six or seven days' age or more a small percentage of the individuals—and usually only a very small percentage—show a tendency to lose the fusiform or more active type of form, and to develop a pear-shaped or rounded, more passive type of form. In most of my culture-series (including sub-cultures), these pyriform or ovoid forms are very infrequent and have to be carefully searched for, even on slides where the ordinary parasites are most abundant. The individuals of this character are generally of medium, or less than medium size, but occasionally are large and massive. Pear-shaped forms are seen in figs. 112–114, that of fig. 113 being from a redpoll culture, the others from different chaffinch ones. The dimensions of these parasites (flagellum excluded), are, for example, 8 μ by 5 μ (fig. 113), and $6\frac{1}{2}$ μ by $3\frac{3}{4}$ μ (fig. 114). Medium-sized ovoid forms are 8 μ by 6 μ (figs. 116 and 117). The large ovoid individual of fig. 118 is 13 μ by 7 μ and has a very long flagellum of 24 μ; the small corresponding form (fig. 115), is 6 μ by 4 μ. Although I have distinguished these parasites as more "passive" forms, it is difficult to know whether to regard them as being about to enter on a "resting-phase," for in all cases where I have observed them in what were normal, healthy cultures, these individuals possessed a flagellum. I may mention here that the only instance where I have found rounded-off parasites which lacked a flagellum was in a culture (original), nineteen days old, which was full of atypical, altered forms (cf. below, p. 696).

This type of parasite, whether pyriform or ovoid, to rounded, is almost certainly to be derived from forms in which the alteration in nuclear position has occurred, and in which the modified method of multiplication, by unequal fission, has made its appearance. Pear-shaped individuals, such as

those of figs. 112 to 114, are probably simply the smaller daughter-individuals which have retained the pyriform shape, instead of taking on the fusiform, more active one. On the other hand, most of the ovoid or rounded forms, especially where they are of medium to large size, would seem to arise from the accentuated trypanomonad type of daughter-individual. Transitional phases can be found, showing different degrees in the retraction of the drawn-out flagellar end and the concurrent reduction or disappearance of the undulating membrane. Thus, both the large and the small ovoid individual (figs. 115 and 118) have still a delicate but distinct continuation of the body along the proximal part of the flagellum, which doubtless corresponds, for the most part, to undulating membrane. And in others of these rounded forms, indications of the original membrane are still afforded by the attachment of the flagellum to the side of the body for some distance, the flagellum curving with it—at times partly curling round it, as it were—before becoming free (fig. 119).

In general these rounded forms of the parasite do not, apparently, undergo division. In most instances where I have observed these forms, they are, as I have mentioned, of small or only medium size, and these never show indications of division. One of my culture-series, however, for some reason or other for which I was unable to account, but which was probably due to some variation in the condition of the culture medium, behaved differently from the usual manner. In this culture a pronounced tendency in the development of the parasites was the production of large, massive forms, which are sometimes ovoid or rounded in shape. Examples are seen in figs. 120–123. The parasites in my preparations of this series (taken when the culture was seven days old) are certainly not degenerate or abnormal; this is clearly shown by a comparison of their structure with that of distinctly atypical or degenerate forms (c f. below, p. 693). There is none of the irregular multiplication of organellæ, nor of the alteration in the cytoplasmic constituents which is apparent in the latter. I consider that the unusually large proportion

of broad or ovoid massive forms in this series was probably due to a greater growth activity than was usually met among the cultural parasites. And just in this case, it is interesting to note, I have found not infrequently various stages of division in ovoid or rounded individuals (cf. figs. 121, 123–125). Making allowance for slight differences due to the more massive form, the process appears to follow, in the main, the unequal method of fission. In all these forms, whether dividing or single, it may be as well to state, the flagellum was present; none of them showed any signs of absorbing or otherwise losing this organella.

The next type of cultural form of the parasites which I have to describe is quite distinct from the preceding ones, being markedly trypaniform. By the term trypaniform is understood the condition characteristic of a trypanosome, where the kinetonucleus lies much nearer to the aflagellar end of the body than does the trophonucleus, and where, consequently, the flagellum is attached by an undulating membrane along the greater part of the length of the body.

In my cultures I have found trypaniform phases, differing slightly in character, at two different periods of the development. As regards one case, I came across this type of the parasites rather accidentally as it were, in the following manner. I inoculated culture-tubes from the chaffinch which had a strong halteridial infection, in addition to small forms of Trypanosoma fringillinarum, in the peripheral circulation. These culture-tubes were examined much earlier than it was my custom to do, namely after forty hours had elapsed. This was not on account of the trypanosomes, as I knew from former experiences that at this early period they would probably not have multiplied sufficiently for me to be able to find an individual on a smear without prolonged searching; it was because I wished to see what development, —if any—was undergone by the halteridia in the culture.[1] In examining a good living drop to see if I could find any halteridial oökinetes, I noticed one or two trypanosomes which

[1] See below, p. 727.

were very active, travelling much more rapidly than was customary in the case of these cultural forms. In the course of looking for halteridia on a permanent smear (made at the same time), I happened very fortunately to come across a trypanosome, and this was so different from the usual trypanomonad type that I subsequently examined my preparations of this series thoroughly to ascertain whether this was the prevailing type. Unfortunately the trypanosomes are very scarce, only three or four on a large film. It is noteworthy, however, that all the parasites seen as a result of systematic searching are in the same trypaniform phase, and show only slight individual variations.

The type is extremely thin and slender, the parasite having a distinctly vermiform appearance (figs. 10, 126, and 127). The body is from 21 to 25 μ in length, excluding the flagellum, and its greatest breadth only from $1\frac{1}{4}$ to $1\frac{1}{2}$ μ. The aflagellar region is very long and finely tapering. The kinetonucleus is far removed from the trophonucleus, and generally lies about midway between the latter and the aflagellar extremity. Its actual distance from this end varies from 6 to 9 μ, depending upon the degree of attenuation. The undulating membrane is in most cases very narrow, and practically distinguishable only by its flagellar border. In some individuals the flagellar border originates, not in close proximity to the kinetonucleus, as is usually the case, but from a point some little distance beyond, i.e. on the aflagellar side of the kinetonucleus (figs. 10, 127). A distinct granule (blepharoplast or basal granule) can often be made out at its commencement. The length of the free flagellum is from 8 to 11 μ. The trophonucleus, instead of being the usual shape, namely, oval or rounded, is considerably elongated in the long axis of the body, this being in relation, in all probability, with the narrow form.

The other instance of the occurrence of parasites of a trypaniform type in my cultures was in a series from a six-day (original) culture of the chaffinch-form, taken when the trypanosomes, of the ordinary, definitive type, were very

scanty in the blood. The parasites are numerous, nearly all being, of course, in one or the other variety of the trypanomonad phase. Exceptionally, however, individuals occur which show the trypaniform condition; for example, on a smear containing between two and three hundred parasites there are four or five such, three of which are drawn in figs. 129–131. I have not found any which correspond exactly to the individuals of this type just described. The parasite in fig. 129 approximates fairly closely to those of figs. 126 and 127, but it is distinctly shorter and relatively not quite so slender. The two other individuals, on the other hand, while altogether much larger, are still very slender in proportion to their length; and in these the aflagellar part is very prolonged and vermiform. While agreeing in general form and character with the parasite, for instance, of fig. 10, they represent, it would seem, an older, later condition. The individual of fig. 130 has attained, probably, the fullest development of this type, at least as far as the culture is concerned; it constitutes, I consider, a most important phase.

The length of the body alone is 36 μ, and its greatest width 2 μ; the distance of the kinetonucleus from the aflagellar extremity is $11\frac{1}{2}$ μ. The free flagellum is only 8 μ long. The trophonucleus of this individual presents a remarkable appearance (fig. 130). The chromatin is arranged in a series of short transverse bars, forming a longitudinal row—hence the description "ladder-like." I have found a quite similar condition in two other examples of this type; but in the other large vermiform individual I have figured (fig. 131) the chromatin is not arranged in such a definite ladder-like manner, but appears to form a fairly regular double row of grains.

None of the trypaniform parasites which I have found—in either case—showed any indications of division.

The types above described include all the cultural forms of the trypanosome observed, which I have no hesitation in regarding as perfectly normal and regular. As I shall mention more particularly later, they are closely paralleled

by flagellate forms known to occur in various blood-sucking invertebrate hosts.

I may now contrast with them certain other cultural forms found, most of which I have equally little hesitation in considering as abnormal or atypical forms, developed by the parasites as a result of unfavourable conditions in the medium. These forms are found in old, original cultures of, say, twelve days or more, in which multiplication has gone on to a very great extent. It must be borne in mind that such a medium no longer corresponds at all to any condition met with in an insectan host. In an insect, the digestion of the imbibed blood—the medium of the parasites—and its absorption are completed in the course of a few days at most; by this time the parasites remaining in the digestive tract have passed into the resting, attached phase. In an old culture, on the other hand, the fluid medium is still present, presumably containing a certain amount of nutriment of a kind, but now considerably altered in character by the addition of waste products of the metabolism of the parasites, which have doubtless a deleterious action on the trypanosomes. In subcultures made at sufficiently short intervals, these abnormal forms are usually not found at all. In this case it is as if the transferred parasites remained continuously in a pure medium, which may be looked upon as a substitute for the medium in the stomach of the insect—at any rate during the early period of digestion.

A most interesting feature of the morphology of these forms is that very few of them show the trypanomonad phase; nearly all the parasites have passed into a more or less herpetomonad-like condition. The earliest indication of an alteration in the character of a culture is afforded by the appearance of such forms. They are to be met with in cultures of ten or twelve days and onwards. At first, of course, these individuals are very few in number.

Examples of this "pseudo-herpetomonad" condition, as I propose to term it, are seen in figs. 140–146; figs. 140, 145, and 146 are from a chaffinch culture of twelve days; fig. 141

is from a redpoll culture of nine days, and figs. 142–144 from one of nineteen days. The body is fusiform to long and slender in shape. The two nuclei are situated distinctly in the flagellar half of the body; they lie usually fairly close together. The appearance of the flagellar end of the body and its relation to the flagellum is in general intermediate between that found in the trypanomonad type and that in a typical herpetomonad form. The flagellum itself is only connected with the body for a comparatively short distance, and is usually not obviously attached along one side of the body to any extent (figs. 140, 141, 143, and 144); hence there are no indications of an undulating membrane. This proximal portion of the flagellum is, in the majority of cases, chiefly intra-cytoplasmic, constituting simply a rhizoplast, and corresponding to the rhizoplastic part of the flagellum in the trypanomonad forms (before it passes to the surface to become the border of the membrane). On the other hand, the flagellar end of the body, while sometimes fairly sharp and acute, approximating to the condition in an ordinary herpetomonad (cf. figs. 140, 141, and 147), may taper more or less gradually (figs. 142, 144, and 145); hence, in these cases, where it is drawn out a little with the flagellum, the latter may be regarded as "attached" for a short—or very short, distance. For this reason, and because the two nuclei are closer together than is customary in a herpetomonad, this condition is preferably distinguished as pseudo-herpetomonad. The difference will be readily understood when it is remembered that all these individuals are derived from trypanomonad forms by the more or less complete loss of the undulating membrane and its attached flagellar border; hence, of course, parasites showing all manner of intermediate stages in the process are to be met with.

In the early formed individuals of this pseudo-herpetomonad variety there is nothing about them to indicate that they are actually abnormal or unhealthy. As I shall discuss subsequently, however, I think it is very probable that the occurrence itself of this unusual condition is the consequence

merely of the unusual environment; I am very doubtful whether it can be regarded as representing a normal phase of the life-cycle. In any case, however, as the age of a culture increases, and these forms multiply and predominate —the trypanomonad phase as quickly declining—numerous irregular forms of the parasites are met with, which are manifestly unhealthy. As might be expected, the form and size of these individuals varies considerably (cf. figs. 145–154, taken either from a twelve-day chaffinch culture or from a nineteen-day one from a redpoll). Some of them are long and narrow, others pear-shaped, while others are large and massive, ovoid, or of ill-defined shape.

The abnormal condition of these forms is particularly indicated by certain cytological characters, which I have never observed in normal individuals. A common feature is the occurrence of a peculiar altered appearance in the neighbourhood of the rhizoplastic part of the flagellum. Sometimes there is a cluster of red-staining granules in this region of the cytoplasm (figs. 145, 146). In the more massive forms there is usually a greater or less amount of a diffuse, indefinite substance, which also stains red. This substance is often more or less streaky in form, one or more streaks commencing in the neighbourhood of the rhizoplast and running backwards in the cytoplasm for a short distance (figs. 150, 151, and 153). In a few individuals the streaky condition is combined with the occurrence of the granules (fig. 152). I am unable, unfortunately, to offer any certain explanation of this interesting character, owing to the fact that I have only had material stained with Giemsa in which to observe it; very likely the appearance is different after other methods of staining. So far as the granules are concerned, they do not differ in their staining reactions from the ordinary chromatoid granules which are often found in normal trypanomonad types; the latter, however, are scattered more or less generally throughout the body, whereas the particular granules under consideration are always concentrated near the rhizoplast. Hence, it is not certain that the granules

have the same significance in the two cases. With regard to the curious streaky substance, its position in relation to the basal part of the flagellum certainly suggests some association with this organella; it seems to me not at all unlikely that its presence is connected with the disappearance of the trypanomonad character, and, indeed, a comparison of figs. 119, 149, and 150 prompts the query whether it may not possibly represent the remains of a flagellar border which has been actually absorbed by the parasite in the case of some of these massive forms.

Another cytological character often apparent in fairly old cultures is vacuolisation. One or two small vacuoles in the cytoplasm may be seen occasionally in individuals of quite regular form; but, on the whole, in my cultures parasites belonging to the definite types recognised above are free from vacuoles. The occurrence of a few small vacuoles in an individual doubtless signifies nothing very abnormal; when, however, the cytoplasm either appears practically full of vacuoles, or else contains one or two huge ones (fig. 154), this ought most probably to be considered as an unhealthy sign.

Very marked indication of a disturbance in the mutual balance of the various cell-constituents is frequently seen in an irregular distribution of the nuclear organellæ. Parasites with two trophonuclei and a single kinetonucleus are not uncommon (fig. 156). These are not to be interpreted as individuals which are in an early stage of division, the process having been begun by the trophonucleus. On the contrary, they are the result of a division in which the nuclei have been unequally apportioned between the two daughter-parasites. This is clearly shown by fig. 157, where the cytoplasm is splitting in such a manner that one daughter-individual has both the trophonuclei and the other only a kinetonucleus. The remarkable feature is that these forms without a trophonucleus can live alone, at any rate for a certain length of time, for I have observed four or five examples in the course of examining my slides of this series (fig. 155). I have never found an active, flagellated form with a trophonucleus

but without a kinetonucleus. In some of the large massive parasites numerous nuclei and flagella are present (figs. 162 and 163), the number of the different organellæ not by any means corresponding. Successive multiplication of the latter has taken place without concurrent division of the cytoplasm; later, the cytoplasm would probably split into three or four portions, and it might very well happen as a result that one of the individuals thus formed would be happy in the possession of three trophonuclei (fig. 158).

Another interesting irregularity in division is met with rarely. This consists in the unequal splitting, longitudinally, of the cytoplasm of certain large individuals, a thin form, with (fig. 159), or possibly without (fig. 161), a flagellum being cut off from the side of the parent. An important point is that these forms have no definite nucleus of either kind—i.e. they are apparently without both tropho- and kinetonucleus. In fig. 161 the individual—if such that portion of the cytoplasm can be termed—about to be cut off has a clump of granules, but that in fig. 159 has nothing at all. I have not observed a narrow form of this kind actually free; in fig. 160, however, an active pear-shaped individual is drawn which also has no definite nucleus, but which possesses many red-staining granules. I have no doubt whatever that these forms are purely "freaks," the result of a degenerative mode of division, and die off quickly after being set free. There is a general resemblance, it will be noted, between this production of enucleate forms, in my cultures, and the formation of sickle-like (so-called "spirillar") forms in cultures of Leishmania donovani, described by Leishman and Statham (8). It is highly probable that, in that case, too, the process is due to an abnormal condition of the Leishmania parasites (which, of course, ultimately degenerate and die off in cultures), and that such forms have nothing to do with any natural developmental phase in the insectan host.

Reference has been made already to the occurrence of rounded forms lacking a flagellum. These have been seen only in an old culture of nineteen days, in which they are not

infrequent. A few are medium in size (fig. 135), but most of them are small (figs. 137, 138, and 139). It is quite obvious from their appearance that these forms of the parasite, in the culture at any rate, are not merely "resting," persistent phases, but are degenerating and dying. And it is interesting to note that the process of degeneration takes place by a gradual disappearance of the nuclear elements. These no longer stand out, sharply stained, in the cell. They lose their distinctive affinity for the stain and become less and less distinguishable from the general substance of the body; at the same time they tend to diminish in size, as if they were being dissipated in the cytoplasm. The last stage of the parasite is an indefinite body, which stains a dull or faint red. Hence, so far as the cultural forms are concerned, all the evidence I have goes to show that the loss of the flagellum means approaching degeneration and death (contrast, for example, the parasite of fig. 136 and that of fig. 138, which are on the same slide and within a few fields of each other).

The above description includes all the different types and the chief varieties of form which I have observed among the trypanosomes in cultures.

Agglomeration.—I have, next, a few observations to make upon the characteristic feature known as agglomeration. I have seen many instances of this occurrence in my cultures. I have never found it in early original cultures (i.e., of less than six or seven days), nor in subcultures. Agglomerated clusters are only met with when the parasites have become abundant in the medium. The clumps are of all sizes, from small ones composed of a few individuals (a dozen or less) up to large masses containing hundreds of parasites. Now and again, in these large aggregations, the parasites are clustered round more than one centre, i.e. in these cases there is an approach to the condition of secondary agglomeration, distinguished by Laveran and Mesnil from primary (single) clusters. In all the clusters seen the parasites have their flagella directed towards the centre of the rosette.

On more than one occasion I have noticed the commencing formation of a clump in a cover-slip preparation of living parasites, where every field contained numerous individuals. Here and there are small numbers of parasites, which have become entangled by their flagella, the distal portions of which appear to be inextricably intertwined.

Once started, the increase in size of cluster may take place in two ways: (1) by the addition of fresh individuals from the surrounding medium, which are continually being attracted; and (2) by the multiplication of forms already present. The increase is undoubtedly due much more to the former method than to the latter; during the early stage, at any rate, it is probably almost entirely due to the accession of more individuals. In short, these clusters are formed mainly by agglomeration. As a matter of fact, dividing forms are comparatively rare in all the clusters I have examined (cf. figs. E–G, Pl. 5). I once left a cover-slip preparation containing a great many free, active parasites for two or three hours; when I returned to it I found several large clumps which had not been there before. It was impossible that these rosettes could have arisen otherwise than by agglomeration; they all had their flagella centrally directed and resembled the cluster of fig. G, except for the fact that some were even larger.

An early stage in the formation of a cluster is seen in the micro-photograph reproduced in fig. E. The individuals composing it differ appreciably in form and size; some of them, at the periphery, had apparently only recently been attracted, and were not yet firmly attached. Only two individuals are undergoing division. The beginning of a secondary agglomeration is instructive. Parasites continue to be attracted to the clump, but owing to the number already present the newcomers are unable to penetrate in between them and become firmly attached. Hence they tend to form a subsidiary cluster for themselves (figs. E and F). The large agglomeration-cluster of fig. G is apparently made up of individuals attached around three centres, two of which, the

older two (in the upper right-hand part of the figure), are partially confluent.

It is important to note that agglomerations are formed of individuals which are of a quite normal type. Nearly all the parasites of the clusters figured, for example, are definitely trypanomonad in character, either fairly long and fusiform, or belonging to the pyriform variety of individual. Agglomerations of less typical forms, pseudo-herpetomonad in character, also occur, but I have not met with them to any extent, even in old cultures.

Novy and McNeal, in their account of cultures of avian trypanosomes (14), make a great point of distinguishing between multiplication rosettes and true agglomeration clusters. They regard all rosettes in which the parasites are joined by their flagella, corresponding, that is, to those I have just described, as arising by successive multiplication from a single individual, which starts the culture. Only those cases, on the other hand, where the parasites are united by their aflagellar ends, are considered to be true agglomeration clusters. Until I myself came to work with cultures, I had no idea but that the view of these authors was correct, and that these two opposite kinds of clusters resulted from quite different processes. Studying Novy and McNeal's description and figures in the light of my own work, I feel sure that these authors have given an entirely wrong interpretation of the clusters, which they regard as multiplication rosettes. Novy and McNeal consider that the whole process starts from a single cell, which is more or less rounded off, and has no flagellum. This gives rise, by division, to a few cells, which now possess flagella; by further multiplication, a typical rosette of spindle-like forms is produced.

Novy and McNeal's figures on Plates 8 and 9, which are from excellent micro-photographs, are most instructive, and are, in my opinion, convincing evidence that the view these authors put forward is incorrect. Most of the figures represent simply clusters, large or small, of different forms

of the parasite, certain of which appear distinctly unhealthy. The authors state that all the figures on the plates to which I am now referring (as well as others) are of parasites from a culture in the seventh generation, grown for seven days, by which I understand them to mean a sixth subculture, itself of seven days' age. This long-continued cultivation doubtless accounts both for the varieties of form present, as well as for the number of clusters. Their fig. 2, Pl. 9, supposed to represent an early stage in rosette-formation, shows a large indefinite-shaped parasite, in which irregular multiplication of the nuclei is going on. There is no indication of the development of any flagella, and I have no hesitation in regarding this individual as an abnormal, degenerating form. That it would ever give rise to a rosette of active, flagellate parasites is most improbable. Again, fig. 3, Pl. 9, represents an agglomeration cluster of four or five somewhat similar forms, three or four of which, however, are not quite so degenerate, as they still possess flagella; but the same irregular multiplication of the nuclei is shown.

Phases such as these have, I venture to say, no connection whatever with the rosettes of more typical parasites figured on Pl. 8. Fig. 2, here, is a small cluster of a dozen pyriform individuals, each with a single, centrally directed flagellum. Not one of the individuals shows the least sign of division. Similarly in fig. 1, Pl. 8, there is a cluster of about eighteen parasites. Hence, in neither of these rosettes is there any evidence that they are going to give rise to one of many more individuals, such as that of fig. 4, Pl. 8, by multiplication. And, from my own experience, I know that such rosettes can be formed very quickly indeed. In this cluster of fig. 4 there are several individuals at the periphery, which are manifestly only loosely attached, and whose flagella cannot be connected with the central core (cf. my own figures). There can be no doubt that these are the individuals which have been most recently attracted to the cluster.

A point in favour of this view of Novy and McNeal's would be furnished by evidence which went to show that two

typical daughter-parasites often remain entangled by their flagella after division. Now, as I have stated, the flagellar ends of the two individuals resulting from division (i.e. longitudinal fission) always become widely separated, and I have never seen any instance of such an occurrence. Even in the rare cases where multiple (quadruple) longitudinal fission is proceeding, the flagella are all distinctly free from one another, and when the cytoplasmic division was completed, the daughter-individuals would doubtless separate. Moreover, from Novy and McNeal's figures, it is obvious that the dividing forms in their cultures behaved in a similar way (cf. figs. 1, 2, and 5, Pl. 7).

Hence, to conclude, I regard Novy and McNeal's rosettes, in which the parasites are attached by their flagella, equally with those in my own cultures, as true agglomeration clusters, originating, and in the main increasing, by the coming together of independent individuals. There can be no doubt, it may be pointed out, that agglomeration of trypanosomes by the flagellar end does occur in the invertebrate host; the process has been described, for instance, in the case of T. lewisi, when in a louse, by Prowazek (22), and when in a flea, by Swingle (33).

On the other hand, there is no reason to doubt that in certain types or phases of the parasite agglomeration in cultures may take place by the aflagellar end; this is stated by Novy and McNeal to occur in the case of their "spirochætes." I have never had cultures which showed a sufficient number of parasites belonging to this type for agglomeration to occur, and so am unable to say more upon this point. It is interesting to note, however, that agglomeration of trypanosomes in the blood of the vertebrate hosts takes place by the aflagellar (kinetonuclear) end, and these "spirochætes" are also definitely trypaniform; in contra-distinction to these, parasites of the trypanomonad type form rosettes which have their flagellar ends attached.

Summary and General Remarks on the Cultural Forms.

From my observations on the cultural forms of T. fringillinarum a few interesting and important data have been obtained, relative to the course of the development of the parasites on passing into the culture-medium. The earliest type of form which I have found is a slender, trypaniform phase. This is soon replaced by the characteristic trypanomonad phase, into which most of the trypaniform individuals pass. This trypanomonad phase is the predominating cultural form, and it is persistent, apparently, so long as the condition of the medium remains healthy. During this period, however, in a culture of six days' age, trypaniform individuals have also been seen, though they were extremely few in number. Further, rare instances of another form have been found, which is distinguished by its vermiform appearance, and by the remarkable ladder-like character of its trophonucleus. This phase is doubtless simply a further development of the ordinary trypaniform type. Whether these later trypaniform individuals represent forms of this character which have been persistent from the commencement of the infection, or whether they indicate a second development of this phase from the trypanomonad type, I have not sufficient evidence to decide. I am rather inclined to think, however, that the latter may be the case; for one or two individuals have been found which might correspond to transition-forms in such a passage (fig. 128).

Since the above research was carried out, I have been studying, in conjunction with Prof. Minchin, the parasites of Athene noctua, and I have observed the early developmental phases of a trypanosome (most probably T. noctuæ) from this bird, in the stomach of the mosquito (Culex pipiens). We hope to publish in due course a full account of this work, but I wish to refer here to one or two general facts. In the first place, to answer any possible criticisms, it may be stated

expressly that the flagellates which I am about to mention were derived, beyond all question, from the little owl.

The parasites occur both in the trypanomonad and in the trypaniform phase. Some of the latter individuals resemble the vermiform type of figs. 10 and 137 closely, the only difference being that the attenuation may be even more pronounced. In fig. 132 is drawn such an example, which shows the extraordinary slenderness of the body. Hence, so far as I am able as yet to compare the two cases, this elongated trypaniform type develops to a much more marked extent in natural conditions than was the case in my cultures; in the latter, for some reason or other, it was soon almost entirely superseded by the trypanomonad type.

The occurrence of anything approaching a herpetomonad phase has only been seen in cultures of a certain age, in which there is every reason to believe the condition of the medium must be becoming abnormal and unhealthy for the parasites. Even then, it is only very seldom that an individual is found which corresponds at all closely to a true herpetomonad (fig. 147); most of the parasites assume what I have called a "pseudo-herpetomonad" condition, which is readily distinguishable from that of an ordinary herpetomonad. With regard to the occurrence of rounded-off "resting" phases, forms of this kind without a flagellum were seen also only in old cultures, full of altered forms, and the individuals which were in this condition were manifestly degenerating and dying. Hence, from such individuals no conclusions can be drawn respecting the occurrence of rounded, aflagellar phases as a normal part of the life-cycle in the insectan host. Such a phase may occur or it may not.

What may be regarded as highly probable, however, is the occurrence in natural circumstances of forms which correspond to the small fusiform or pyriform individuals of the culture (cf. fig. 111) in an attached condition, i.e. with the flagellum more or less shortened or retracted, and serving as fixative organella. The predilection that such forms have for forming groups or clusters in the cultures (cf. fig. G, Pl. 31, and

also Novy and McNeal's figures of so-called multiplication-rosettes on Pl. 8) is probably to be regarded, indeed, as indicating the tendency of these forms to become attached, when in the natural insectan-medium. In the culture-medium, however, there is nothing for them to attach themselves to, excepting these commencing clusters of their fellow-individuals. Hence, the probable explanation—in great measure, at any rate—of the clumps or clusters which have their flagella centrally directed, is that they represent the attached phase in the insect. This is of well-known occurrence, both among trypanosomes (cf. Prowazek [l.c.], figs. 53 and 54), and among insectan flagellates (cf. especially Patton [**16**, Pl. 9, fig. 22], where a number of Crithidia sp., in Gerris are clustered around a food-particle, and again, Swingle [**32**], who states that a rosette of Crithidia in the sheep-ked, Melophagus, may be formed around a free epithelial cell). In the case of parasites in cultures, when one, two, or three individuals have become entangled by their flagella, the interlocked ends furnish doubtless the "nucleus" for the attachment of many other parasites, with the result that a large cluster is soon formed.

An important point brought out decisively by my cultures is that this avian trypanosome does not proceed to form rounded-off, resting phases immediately on passing from the vertebrate host into the cold medium. And further, I may mention, there is not the least indication of any such behaviour in the case of the trypanosome of the little owl when it passes into the stomach of the mosquito.

Up to the present only one or two accounts of cultural forms of trypanosomes have been published which describe and make any attempt to distinguish between the different types of form and phases developed at different periods in the culture. Of these, the most important for purposes of comparison with my own results is the paper of Novy and McNeal, to which reference has been made. In this connection it must be emphasised that most of the authors' figures of cultural forms (and apparently their descriptions also) are

based upon the parasites present after cultivation has been continued for some time, i. e. in sub-cultures of the sixth or seventh generation, when the culture was fully developed and "enormously rich in flagellates." In such cultures of a trypanosome, regarded by the authors as T. avium, the great majority of the trypanomonad forms were found in clusters, some of which were large enough to be visible macroscopically as patches in the medium. The interesting point is that parasites in the form of "spirochætes" were of common occurrence, sometimes abundant; "spirochæte," it may be as well to state, is the term applied by Novy and McNeal —somewhat misleadingly—to individuals of the slender, trypaniform type, similar to those seen in my figs. 10, 126, and 127.[1] These trypaniform individuals were mostly free, very active, and some were undergoing division.

Hence the condition found by Novy and McNeal obviously represents a much later period in the development of the culture than any I have described above, and I cannot find any account of the early course of the development, i.e. during the first five or six days or so. The authors do not say at what intervals of time their sub-cultures were made, but it is evident, from the number of the "generation" given, that the trypanosomes must have been cultivated for at least some weeks. In the case of T. fringillinarum, I was unable to obtain any development in my cultures corresponding to that found by Novy and McNeal in T. avium. If I did not sub-culture frequently enough the parasites become abnormal and degenerative, so that a preparation would show nothing but altered, pseudo-herpetomonad forms and so forth, and when I sub-cultured frequently the trypanosomes retained, for the most part, the trypanomonad phase. I never continued subculturing for so many generations as Novy and McNeal did; it is only since I have come to study carefully my preparations and to compare

[1] Although in one or two cases these parasites show indications of an extended nucleus, in no case is a definite ladder-like appearance figured or described.

my results with those obtained by Novy and McNeal that I realise some additional knowledge might have been gained by continuing to cultivate longer. In one case I subcultured four times at fairly slow intervals; this was done chiefly with a view to seeing how long I could keep a culture of the trypanosomes alive (cf. above, p. 647). Unfortunately, being kept away for a few days by ill-health, I missed an opportunity of examining this fourth subculture at a time when the parasites would have been very numerous; and before my return an unfortunate accident had terminated their career. Possibly this subculture might have shown more trypaniform individuals.

Novy and McNeal go to the length of founding two new species of Trypanosoma upon the different behaviour and appearance of certain of their cultural forms. In fact they distinguish several types or varieties chiefly or entirely upon a basis which is most inadequate and misleading, namely, on a comparison of the multiplication-rosettes (really the agglomeration-clusters) and the free "swarming" parasites in the different cases. I only wish to point out here that, in the case of both their new species, viz. T. laverani and T. mesnili, the free-swarming forms which they compare with the slender, trypaniform type of the other species dealt with (T. avium) and contrast with the rosette-forms, are in reality not trypaniform ("spirochætes") at all, but are ordinary trypanomonad forms, which do not differ essentially from those constituting the rosettes. This is perfectly obvious from a comparison of their figures on Pls. 5–7.

The matter amounts simply to this: In the case of these two species, the authors have not got a development of the trypaniform type at all. Many of Novy and McNeal's figures of these forms, especially of T. mesnili on Pl. 6, are of individuals which show pronounced vacuolisation, and which, in my opinion, appear distinctly unhealthy; also the cluster of individuals of T. laverani, reproduced in fig. 3, Pl. 7, I regard as partly composed of abnormal forms. In short, from a comparison of the figures given of T. laverani and

T. mesnili with most of those of T. avium, I am strongly inclined to say that the cultural development of the former parasites was not proceeding so successfully—at any rate, when the preparations concerned were made—as that of the last-named species.

Slight differences in the constitution of the medium may certainly influence the rapidity of growth of these cultural forms, as I have stated above, and probably also, to a certain extent, the manner of their development. Further, it is quite likely that different species of trypanosomes, when cultivated in the same medium, may also differ in their rate of growth and in the development of the different types of form. Hence, I think we may agree with Novy and McNeal, although on quite different grounds, that the parasites which they name T. laverani and mesnili are at any rate different from the other (T. avium). Moreover, it may be reasonably inferred that under slightly different conditions—in one way or another—of the medium, these forms would also develop a trypaniform phase. For it will be seen from the subsequent context of this paper that there is every reason to suppose such a phase is of regular occurrence at some period in the development of a trypanosome outside the vertebrate host. As a matter of fact, T. laverani itself appears to be very closely allied to the trypanosome with which I have been working.

The only other paper dealing with cultural forms, to which I need refer is a note by Thomson (35), on the cultivation of trypanosome (probably T. danilewskyi), from the goldfish, which gives instructive indications of the course of development of that parasite in cultures. It is most interesting to find that there is a general resemblance between the course of events in the case of that piscine form, as outlined by Thomson, and in the avian parasites discussed above. Thomson does not describe any developmental forms occurring earlier than the seventh day. By this time the parasites are in a phase corresponding to my accentuated trypanomonad type; and division by a quite similar method of unequal

fission is taking place, a small fusiform or pyriform individual being cut off from the large, more or less club-shaped parent-form. Several of Thomson's figures are, indeed, almost identical with some of my figures. Another important point is that distinctly trypaniform individuals were present, and such forms were found to be more frequent later on, for instance in a culture of the forty-second day.

As Thomson says, it is probable that earlier phases in this development might have been found before the seventh day. It is interesting to note that Thomson figures an unaltered trypanosome (as it left the blood of the fish) in the culture of seven days. Thomson's view is that the large, club-shaped trypanomonad individuals are derived directly from such trypanosomes by an alteration of the body-form, most of the protoplasm becoming concentrated in the aflagellar part of the parasite, which thus becomes greatly swollen in appearance. According to Thomson, there is no prior multiplication of the parasite in an ordinary trypanomonad condition. Hence in this case a type of form very similar to that which I have found in my cultures (cf. figs. 97, 98) is attained by a quite different process; in the culture of the avian parasites, the trypanosome-phase is quickly lost and active multiplication in the ordinary trypanomonad phase goes on.

It is evident from this that the development of the piscine type in cultures proceeds much slower than that of the Avian form, and this bears out, in an interesting manner, the facts so far known relative to the development of the two types in the true invertebrate hosts (leech and insect) respectively.

The Significance of these Cultural Forms of Trypanosomes in Relation to the Question of an Alternate Invertebrate Host.

When we come to compare the chief types of form described above as occurring in cultures of trypanosomes from different vertebrates with the flagellates described by various authors from blood-sucking invertebrates, which they have considered

as being phases in the life-cycle of some vertebrate trypanosome, we find at once a fundamental resemblance, while in one or two particular cases there is a strikingly close similarity in detail. It would occupy too much space to follow out this comparison at length. I must content myself with a reference to various papers, and with a few indications as to the chief points of agreement.

It may be noted, as a preliminary, that I follow Patton's definition of, and distinction between, a herpetomonad form and a crithidial or trypanomonad form; the terms "crithidial" and "trypanomonad" are practically interchangeable, but I prefer to use the latter, at all events when referring to this phase in connection with a vertebrate trypanosome.[1] Further, it is necessary to emphasise the fact that the characterisation of these two types is based upon their structure when in the active, extended, flagellate condition; in other words, the diagnostic form of the parasites is only seen when they are in this condition. Rounded, resting phases, whether possessing a flagellum or lacking one, cannot be regarded by themselves as representing either a herpetomonad or trypanomonad phase, simply because, when the parasites are in this condition, the features used for distinguishing between the two types are not present. It is certainly due to Patton that we are at last able to realise that there are these two perfectly definite types, a herpetomonad and a crithidial or trypanomonad one, and to distinguish clearly between them. Until Patton separated the two types upon the above basis, the greatest confusion often prevailed as to whether a given parasite belonged to one or the other; and it must be admitted this confusion was chiefly due to the unsuitable diagnostic characters used by Léger in his earliest descriptions of these forms.

The memoirs in question are those by Miss Robertson (**23**, **24**, and **25**), Minchin (**11**), Prowazek (**22**), Stuhlmann (**31**), and Roubaud (**26**). In all the parasites described, namely, T.

[1] There have been, hitherto, two quite different meanings attached to the term "crithidial" (cf. also below).

raiæ and T. vittatæ (Miss Robertson), T. grayi (Minchin), T. lewisi (Prowazek), T. brucii (Stuhlmann), and T. gambiense, cazalboui, and congolense (Roubaud), a trypanomonad phase occurs, and is usually prominent. In all of them a definite trypaniform phase (i. e. one in which the kinetonucleus is some distance on the aflagellar side of the trophonucleus), is also met with. And in two cases, namely, T. brucii in Glossina fusca (Stuhlmann), and T. raiæ in Pontobdella (Miss Robertson), the occurrence of a greatly elongated trypaniform type with an extended, ladder-like nucleus is described. These are the only cases of which I know where this characteristic type of form has been seen in an invertebrate; and it is highly significant, I think, that a similar form occurs, beyond all question, as a developmental phase of more than one avian trypanosome. Unfortunately I am not yet able to add anything to our knowledge of the purpose or meaning of this interesting form, which has been variously considered as possibly a male form, and—more likely—as a propagative individual infecting a vertebrate host.

The same close agreement holds good also for another important point, namely, the absence—apparently the entire absence—of anything corresponding to a true herpetomonad phase in these parasites when in the Invertebrate host. Out of a total of some hundreds of figures in the above memoirs, there is not one which shows a typical herpetomonad individual, such as, for instance, Herpetomonas muscæ-domesticæ, lygæi, jaculum, etc., or Leishmania. There are only one or two figures, e. g. in one of Miss Robertson's accounts (**24**, figs. 12, 21, and 22), which could be regarded as in any way approaching a herpetomonad condition; and it is precisely in such a case, moreover, that the essential proviso noted above must be borne in mind. The individuals figured are manifestly intermediate stages in the development from a rounded resting-phase to an active flagellate type of form. Further, they are all dividing, and one of the daughter-individuals (fig. 21, right-hand side) is already acquiring the

trypanomonad condition. Hence these cannot be regarded as representing in themselves determinative phases, but are rather only transitory stages in the development of a trypanomonad (or it may be a trypaniform) type, such as is exemplified in most of Miss Robertson's figures of active, flagellate individuals. On the other hand, what is far more important is that none of the numerous elongated "monadine" forms figured by Roubaud (26) show any indication of herpetomonad affinity. Last, but not least, the so-called herpetomonad forms of T. grayi—the extremely slender ones, which proceed to encystment—have nothing whatever to do with the herpetomonad type, as indeed Patton has already pointed out, but are unmistakably of the trypanomonad type. This mistake arose, of course, simply by following Léger's mode of distinguishing between the two types chiefly by means of the body-form.

There can be no doubt, I think, that this briefly outlined comparison enhances the probability that the various accounts to which I have alluded do actually relate to phases of the life-cycle in an invertebrate host of the different vertebrate trypanosomes which they purport to do; in my own opinion, and in that, I venture to say, of most other people, the matter is certain.

I should like to offer a few further remarks upon the still disputed question of a vertebrate trypanosome in its alternate host *versus* a natural flagellate of the invertebrate. In the first place, two classes of invertebrates are principally concerned, namely leeches and insects. The former I intend to leave altogether out of account, as up to the present not the slightest evidence has been brought forward of the occurrence of any flagellate parasites in this class of hosts, which are not developmental forms of some vertebrate trypanosome. In the case of insects the subject is much more complicated; since in many non-blood-sucking insects flagellates occur which can be only parasites of the one host.

As a result of the above comparative observations, one general proposition can be stated, I believe, which ought to

prove of considerable help in this connection. It is this: Parasites exhibiting a trypaniform condition in a blood-sucking insect must be considered as belonging to the life-cycle of a vertebrate trypanosome, until the contrary is definitely established; and the onus probandi lies with those who maintain the opposite view.

Another conclusion which appears indicated is that, in general, such parasites do not pass into a true herpetomonad condition; in other words, they have not a definite herpetomonad phase in the life-cycle. Bearing in mind that many, at any rate, of the vertebrate trypanosomes which have an insect as their alternate host are almost certainly to be derived from a herpetomonadine form, which was originally a parasite solely of the insect, it will be understood, of course, that in certain circumstances the parasites may revert, as it were, to a psendo-herpetomanad condition, or even to a herpetomonad one, as I have found in the case of my avian trypanosomes in cultures. But with this qualification, all the observations so far recorded point to the above conclusion.

As a matter of fact, the occurrence of typical herpetomonad forms in blood-sucking insects has not been described in nearly as many cases as would appear, at first sight, to be the case. In many of the papers that I have seen which profess to describe such forms, a study of the figures shows that the authors have been dealing really with trypanomonad (crithidial) forms; these are merely further instances of the confusion formerly existing in regard to the diagnosis of these two types. Thus the Herpetomonas algeriense described by the Sergents (**28**) from Culex pipiens does not appear to have anything in common with a true Herpetomonas; from the figures given it must be regarded as a trypanomonad form.[1]

[1] Instances, on the other hand, of what are apparently true herpetomonad forms occurring in mosquitoes and restricted to this host are given by Patton ('Brit. Med. Journ.,' 1907, ii, p. 78) and also by the Sergents (l.c.); but there is not likely to be any difficulty in distinguishing such parasites from phases of a vertebrate trypanosome. I may

Again, Novy, McNeal, and Torrey, in their paper on the flagellates of mosquitoes (15), distinguish two parasites, namely, Crithidia fasciculata and Trypanosoma (Herpetomonas) culicis. These authors also followed Léger's unfortunate definition of a Crithidia, restricting the name to small oval or pyriform parasites with a truncated flagellar end and a short flagellum. The whole objection to this definition lies in the fact that such forms are merely resting or attached phases (in natural conditions) of either crithidial (trypanomonad) or herpetomonad forms. However, in the case of their Crithidia, the figures given show that, in a more elongated condition, it conforms on the whole to the trypanomonad type. Similarly, their other parasite, Trypanosoma (Herpetomonas) culicis, also has a well-marked trypanomonad phase, as, indeed, is implied by the generic position which the authors assign to it; apparently it is placed in the sub-genus Herpetomonas because of its monadine form. I may observe here that these papers by the American authors have been most difficult for me to comprehend, because the indications afforded or suggested by their plates often appear to be opposed to the account given in the text. I have only really grasped the significance of their first paper on avian parasites and their cultural forms since working on my own birds and cultures; and I am sure, from the interesting plates of mosquito-parasites in the authors' second paper, that a further study of the phases and forms which they figure is essential to a correct understanding of their significance. Hence I do not propose to criticise them further at present.

This much, however, must be said in regard to all these cases of the occurrence of trypanomonad forms in mosquitoes. It is at least quite as likely that the flagellates observed were phases of vertebrate trypanosomes—say of avian forms—as that they were purely insectan parasites. I have referred

say here that in the development of T. noctuæ in Culex pipiens I have not come across the slightest indication of a herpeto monad phase.

above to the undoubted indications I have obtained that a trypanosome of the little owl undergoes developmental phases in Culex pipiens. There is, therefore, no reason whatever to doubt any longer that some, at all events, of the flagellate phases described by Schaudinn in mosquitoes which had fed on infected birds were also actually phases of Trypanosoma noctuæ. Moreover, in regard to Crithidia fasciculata itself, the type-species of that unfortunate genus, no one has yet shown that it is solely an insectan parasite. In first describing it, Léger very wisely admitted the possibility that it was only a phase of a vertebrate trypanosome, and this still remains the most logical assumption with regard to it.

Similarly with regard to crithidial forms in other blood-sucking insects, e.g. C. tabani, Patton (18), C. melophagia, Swingle (32), etc., by far the most likely and reasonable view is that these parasites are merely the trypanomonad forms of a trypanosome.[1] One or two cases have been described, however, of the occurrence of crithidial forms in what are alleged to be non-sanguivorous insects, e.g. C. gerridis from Gerris fossarum, Patton (16); such parasites may apparently be regarded as true Crithidia, by which we may understand flagellates that have developed a trypanomonad condition, but which are restricted to an invertebrate host.

Two or three parasites have recently been described, and, moreover, from non-biting insects, which have been regarded as "trypanosomes." They are Trypanosoma drosophilæ, Chatton and Alilaire (3), and two peculiar herpetomonad forms termed Leptomonas mirabilis, from Pycnosoma putorum and L. mesnili, from species of Luculius, which

[1] As regards C. melophagia, I have quite recently obtained evidence which makes this almost certain. After prolonged examination of the blood of a sheep on which were "keds" infected with this parasite, I had the good fortune to find a typical, active trypanosome. This is the first occasion, so far as I know, of a (natural) trypanosome having been found in this domestic animal. There can be little or no doubt that the "Crithidia melophagia" is simply a developmental phase of this sheep-trypanosome in its alternate, insectan host.

have been described by Roubaud (26). In the case of the first-named, the individuals figured certainly appear to be in a definite trypaniform condition, possessing a distinct, though narrow, undulating membrane. The two other parasites are very remarkable, in that typical herpetomonad forms appear to have also a "trypanosome" phase in their life-cycle, and all intermediate conditions between these two extremes are figured. So far as I can judge from the figures given, however, the so-called "trypanosome" phases do not represent a true trypaniform condition in the sense in which it has been understood in the above pages. To begin with, the flagellar end of the body is not drawn out at all, but the flagellum emerges straightway from it. The kinetonucleus is, indeed, near the aflagellar end of the body; but in all cases the course of the flagellum, from the point where it comes into contact with the cytoplasm up to the kinetonucleus, is shown running through the middle of the cytoplasm; it is never drawn lying at the side, still less as showing any undulations. I think this is an important point, and one which tells very much against the presence of an undulating membrane in these Leptomonas. For in the great majority of preparations of trypaniform parasites, however attenuated they may be, and however narrow the membrane, the attached flagellum lies nevertheless at one side (cf. my figs. 10, 126–132, and also Minchin's figures of T. grayi). I think, therefore, that in these peculiar phases a considerable part of the flagellum is intra-cytoplasmic, forming, as it were, a long rhizoplast, consequent on the passage of the kinetonucleus to the opposite end of the body. These forms appear to be quite distinct both from ordinary herpetomonad parasites and from the true trypaniform type. "T" drosophilæ, on the other hand, appears to exemplify the trypaniform condition.

The above summary represents, in my opinion, the present position of this difficult problem of the flagellates occurring in blood-sucking invertebrate hosts. My view on the subject

is the same as that I have maintained in my article on the Hæmoflagellates in Lankester's Protozoa (**39**), as will be seen by anyone who cares to compare that account with the above pages. As a matter of fact, there is now no doubt whatever that one of Schaudinn's far-reaching conclusions was correct, namely, that vertebrate trypanosomes undergo a definite part of their developmental cycle in an invertebrate host, and that true cyclical infection occurs by means of the latter; for conclusive experimental proof has been recently brought forward by Kleine, Bruce and others, Minchin and Thomson. To indicate the work of these authors, however, would be going outside the scope of this paper; moreover, in this discussion, I have preferred to limit myself to the above comparative observations, since most of them provided material on which I relied for support in my article (l.c.).

Patton has of late occupied himself in reiterating his view that in all those instances considered above, as well as in every other case where an author has purported to describe phases of a trypanosome in an invertebrate, the parasites in question were merely natural flagellates of the invertebrate, which had no connection with a vertebrate host. Patton's view is that of scarcely anyone else; even Novy and McNeal have not gone quite so far in this wrong direction. I do not intend to argue the matter with Patton; a perusal of his recent papers suggests that he is unable to appreciate argument. In his latest review (**20**), Patton has adversely criticised, in somewhat forcible terms, my article in Lankester's treatise, chiefly because I have maintained the opposite view to himself. I do not think it necessary to reply at length to Patton's remarks; it is obvious that Patton is hopelessly biassed, and in one or two places I consider he oversteps the boundary of legitimate criticism. I venture to say, however, in justice to my editor as well as to myself, that if a student of tropical medicine and protozoology follows Patton's judgments on our knowledge relating to the hæmoflagellates and their allies, as set forth in his "critical" review, he will obtain a distinctly erroneous and misleading

impression of the group, and one which is further from the truth than the views expressed in my article.

(c) Notes on Nuclear Cytology and Division.

My material, having been all stained by the Romanowsky method, has not proved very suitable for a study of the minute structure of the nucleus (trophonucleus). Nevertheless, in the light of the interpretation which Minchin and Woodcock (**13**) have shown is to be placed upon the "Giemsa-picture" of the nucleus of a trypanosome, I am able to say that, in the case of many, at any rate, of the parasites observed, the type of nuclear structure certainly agrees with that described in that paper. Unfortunately, in the parasites figured from the blood of the bird, the nucleus often shows the usual granular appearance; now and then, however, the definite clear region can be seen, corresponding to the central, plastinoid part of the karyosome, which contains a deeply staining granule in the middle—the intranuclear centrosome (figs. 30, 34, and 51). For some reason or other cultural forms show this appearance, which is to be regarded as the typical one, much more frequently, indeed quite regularly (figs. 7, 8, 72, etc.). The trophonucleus of the individual in fig. 3 is in an interesting condition; it is more faintly stained than usual, the nuclear sap apparently containing little or no chromatin (cf. the numerous chromatoid granules scattered in the surrounding cytoplasm). Whether the deeply-stained central body represents in this case a small karyosome or a greatly enlarged central granule, it is impossible to say. Other instances of an unusual appearance of the trophonucleus are seen in the parasites of figs. 38 and 39; here there appear to be a certain number of separate chromatic masses, of varying size. This condition perhaps represents a fragmentation of the single large karyosome usually present.

The blepharoplast, or basal granule, at the proximal end of the flagellum is sometimes visible in the parasites from the

blood (figs. 4, 28); but frequently the proximal, rhizoplastic portion of the flagellum is not well stained, and then the blepharoplast cannot be made out. In preparations of cultural forms it is generally conspicuous, and now and again very prominent (figs. 10, 71, 81, etc.).

As regards the details of commencing division, the trypanosomes in the blood have provided me, as already stated, with hardly any indications at all. On the other hand, I have obtained a nice series of stages among the cultural parasites. The first act in the process is apparently the division of the blepharoplast at the base of the flagellum (fig. 120). This is followed by the splitting of the flagellum for some distance, which may be fairly short or fairly long (figs. 100, 104, 121, and 123); the splitting never extends, however, throughout the whole of the attached part of the flagellum. In the case of this avian parasite, the splitting-off of a portion of the old flagellum to form the foundation of the new daughter one appears to be of general occurrence. I have observed nothing which would indicate that the daughter-flagellum is formed as an entirely independent outgrowth from the second blepharoplast. Fig. 89 shows a flagellum caught in the act of dividing, the proximal portion being drawn out transversely, as a broad band, prior to splitting. In figs. 88, 100, 104, and 123, the newly formed part is still connected at its tip with the old flagellum; and in fig. 121 the new portion, in this instance only short, has just separated. Of course, once the rudiment, as it were, is cut off, its further growth is quite independent.

The division of the nuclei may begin while the splitting of the flagellum is proceeding (figs. 104, 123), or it may be delayed until the latter process is completed (figs. 90, 121); there is apparently considerable variation in this respect. The first stage in the division of the trophonucleus is most probably the division of the intra-nuclear centrosome, which acts as a division-centre; this is clearly shown in fig. 88. All that can be said from Giemsa-stained preparations as to the rest of the process is that the nuclear substance becomes

extended in a direction more or less transverse to the long axis of the body, this being doubtless brought about by the separation of the daughter-centrosomes (cf. fig. 99); the two centrosomes remain connected by a fibril, which at a later stage may become considerably drawn out (figs. 124, 125). The nuclear material becomes aggregated around these two division centres; as the latter continue to separate, it is pulled out more or less into the form of a dumbbell and finally constricted into two halves, the daughter trophonuclei. With regard to the division of the kinetonucleus, the process, so far as can be judged from the phases seen in figs. 101 and 104, appears to be similar to that occurring in the division of the other nucleus. A distinct thread or band connects the separating halves; this probably indicates a fibril, corresponding to the other, which may also have its terminations in two intra-nuclear division-centres. If this is really the case, not only the trophonucleus, but also the kinetonucleus, possesses an intra-nuclear centrosome.

(D) Comparison of Trypanosoma fringillinarum with other Avian species.

The reasons which have led me to consider all the manifold forms of the trypanosome met with as belonging to one and the same species have been given at the commencement of the description of the parasites, and also alluded to elsewhere in the account, so that I need not recapitulate them here. This illustration of the very great polymorphism which may be shown by one species is most instructive. If, for instance, only two types of form, at opposite extremes as regards size, had been observed, it might readily have been supposed that two different trypanosomes were concerned. And there can be no doubt that many observers, not only of avian parasites but also of others of cold-blooded vertebrates, who have based their descriptions on casual observations of the parasites, have fallen into such an error. So long as the mammalian forms, and among these chiefly the lethal ones, with their comparatively modest variations in form and size, remained

those with which research was principally occupied, the possibility of such striking polymorphism was insufficiently recognised. It is evident, I think, that the safer plan for workers on these naturally occurring trypanosomes will be to regard all the forms met with in any one host as belonging to one species until they have satisfied themselves that this is not the case.[1]

On the other hand, for the purpose of distinguishing different species of trypanosomes, I certainly continue to think that what may be called the biological consideration is, in the present state of our knowledge, the most reliable and useful guide. By this I mean that the less closely related, zoologically, two hosts are, the greater the probability that their trypanosomes are distinct species. As a general indication it may be said that the same parasite may, in certain cases, be parasitic in different species of host, or even in closely allied genera,[2] but where the hosts in question belong to different families, or still more, to different orders, it may be safely assumed, as a working rule, that their trypanosomes are distinct species. The best practical test for this criterion is, of course, the production or non-production of cross-infection.

In making use of resemblances or differences in morphology in comparing two trypanosomes, I think the ordinary adult form of the parasite furnishes the best indications. Take the case of T. lewisi, for example; neither the young daughter-individuals resulting from multiple fission, nor the large, stout, multiplicative individual itself is regarded as the definitive form, the form of every-day occurrence, as it were. Now I think we can carry this comparison very usefully to other cases. Small, fusiform, or stumpy individuals are more

[1] I consider, for instance, that Wenyon (37) has done wisely in including the quite different types of form found, on the one hand, in the guinea-fowl (Numida) and, on the other hand, in a lizard (Mabuia), under one species in each case, viz. T. numidæ and T. mabuiæ.

[2] In this connection attention must be paid to the question of distribution.

likely to be young forms; these may, perhaps, themselves undergo division, as in T. lewisi, and, moreover, in many cases, owing to a slow rate of growth and increase in size, these small forms may give the impression of being distinct parasites. On the other hand, very large, massive forms are likely in many cases to be essentially multiplicative individuals. Of course the possibility must not be overlooked that, in some cases, large, stout forms may be sexual (female) individuals, but up to the present evidence pointing to the occurrence of sharply differentiated sexual forms is only forthcoming in a few instances. At any rate, so far as T. fringillinarum is concerned, I think there is a general parallel with T. lewisi in regard to the different types.

In the case of many of the avian species so far described, the account has been based in all probability upon the ordinary adult type, e. g. T. avium, as emended by Laveran, T. paddæ, Thironx, etc. But in other cases, where only stumpy forms have been described, such as T. hannæ, another T. sp. from Senegambian birds,[1] and T. laverani, these probably do not represent the definitive type. Passing on now to compare T. fringillinarum with certain other trypanosomes, we may begin with the type-species, T. avium. This name was originally given by Danilewsky, who followed his own methods of nomenclature, to trypanosomes found both in owls (sp. indet.) and in a roller-bird (Coracias garrula). Laveran (6) has rightly restricted this specific name to a parasite from an owl (Syrnium aluco), which he considers to be the same form as that observed by Danilewsky; the other trypanosome, from the roller-bird, is in all probability a different species. T. fringillinarum, while showing a general similarity in size and form with T. avium, as described by Laveran, differs in two respects,

[1] This parasite, described by Dutton and Todd (4), occurred in a bird (Estrelda) in which the very different form T. johnstoni was found. It is not at all improbable, I think, that T. johnstoni is the ordinary form, and the broad, stumpy parasite a multiplicative form, of one and the same species.

namely, in the length of the free flagellum, which is much shorter, and in the appearance of the aflagellar end, which is more elongated and attenuated. In addition, the hosts are, of course, quite different in the two cases.

Novy and McNeal have included in the species T. avium a number of parasites they have found in various North American birds. They distinguish two chief forms, viz. large and small parasites, each of which shows considerable variations in size. How Novy and McNeal have been able to ascertain any details with regard to form and size, if they had not better preparations to study than those from which their excellent photos have been taken, it is impossible to say. From their photos of the parasites in the birds, it is obvious that the trypanosomes were wretchedly fixed and stained; in scarcely any can the length of the flagellum or the true nature of the aflagellar end be made out. Hence, any real morphological comparison is out of the question. In any case, on the grounds of occurrence and distribution, it is very improbable that any of the parasites represented the true T. avium. This has been recognised by Lühe (9), who has placed all these forms identified by the Americans as T. avium in a new species, T. confusum—a very apt name. I do not for a moment suppose, however, that all the forms described belong to one species. Novy and McNeal rely partly on the cultural characteristics shown, which they say were similar in all these cases. All their photos of cultural forms of this group of trypanosomes are taken from preparations of a single culture, from one bird only. I should prefer to see figures of cultural forms from the other birds first of all.

The trypanosome which Novy and McNeal distinguish as T. laverani, from an American goldfinch, Astragalinus tristis, is most probably closely related to T. fringillinarum, although I am hardly inclined to think the two forms are identical. The authors only figure a solitary example from the blood, which, from the size given, and from what can be made out from the photo, agrees very well with the small, fusiform individual of T. fringillinarum. There is a

general agreement also, both in regard to appearance and size, between the trypanomonad forms in cultures. The reason which weighs most with me in keeping the two parasites distinct is the different hosts and their different distribution. Unfortunately Novy and McNeal do not describe, as I consider, the definitive type of the parasite, and so I am unable to compare it with that of T. fringillinarum. Other reasons are that T. laverani is said to have a very sparse and slow growth in cultures, and the cultural forms themselves show very generally a peculiar rod-like structure near the aflagellar end of the body. I have certainly never seen this feature in any of the cultural forms of T. fringillinarum.

5. Note on Halteridium fringillæ (Labbé).

I have already published a short paper (38) relating to the chief features of interest which I have observed in connection with this parasite; and I do not propose to repeat in detail the description there given. I wish, rather, to add here a few general remarks and comments.

I am now able to publish many of the actual drawings from which the text-figures in my previous note were made; and these—especially the coloured figures—bring out certain distinctive points very clearly. It is particularly in such a case as this, I may say, that the value of the different tints and depths of colour, produced by the Romanowsky (Giemsa) stain, is apparent. Firstly, in regard to the dimorphism of the nuclear constituents (cf. especially figs. 14, 15, and 17). The smaller nuclear body, representing the kinetonuclear element, is seen to be quite distinct in its staining reactions from the larger body, the ordinary nucleus. These two nuclear portions correspond closely in appearance (leaving out of account the marked difference between them as regards size) to the trophonucleus and kinetonucleus of a trypanomonad parasite, where these two organellæ are close together or in contact.

Again, with respect to the so-called "indifferent" individuals, which are very scanty in number, compared with the female or male forms, figs. 15, 17, and 64, show the characteristically clear cytoplasm, not at all granular, and staining very faintly, of these individuals—readily distinguishable from the granular, deeply staining cytoplasm of female forms.[1] Further, in most of the parasites of this kind which I have found, the kinetonuclear element is relatively large, and may approximate in size to the other nucleus (cf. fig. 64). What exactly is to be understood by the term "indifferent" as applied to these forms, and what their significance is, it is difficult to know. If they are neither male nor female they are not gametocytes; that much is obvious. At the time when I wrote my earlier note on this Halteridium, I was strongly inclined to think that these neutral individuals passed, in certain conditions or circumstances, directly into small trypanosomes. Unfortunately I have not been able to obtain any more evidence in support of this view, either from a renewed study of my own preparations of the chaffinch parasite, nor—which is even more important—from the study undertaken of Halteridium noctuæ, so far as this has yet progressed. Hence the meaning of these "indifferent" individuals, which certainly appear to be quite distinct from the forms of male or female character, has still to be ascertained. I have never found indications of division in them, any more than in the other types.

In fig. 16 is drawn one of the two or three instances I have observed of the remarkable form of individual occurring free in the blood-plasma, which shows a conspicuous line running down the greater part of the body, near one side. This line stains distinctly red, like a flagellum; it appears to start in close proximity to the nuclear masses, and ends in a definite granule. The pigment-grains in this parasite are all aggregated together near one end of the body—that farther away from the nuclei. I regarded the halteridia in this phase

[1] Of course there is no possibility of confusing these forms with male gametocytes, which have a large, diffuse, pale-staining nucleus.

as being about to pass actually into little active trypanosomes, in a manner similar to that described by Schaudinn, that is to say, by getting rid of a portion of the cytoplasm containing the effete pigment-grains and by the development of a flagellum, the proximal, attached part of which constituted the flagellar border of an undulating membrane. In spite of much searching, I have not succeeded in finding any further stages in this developmental change. I cannot suggest any other satisfactory explanation of this peculiar structure, however, and I still continue to think it has some connection with a flagellar development, as will be seen in a subsequent paragraph.

The halteridial parasites of small or intermediate size, which I have now found to occur occasionally free from the corpuscles (cf. p. 663), seem to be quite ordinary in character and show nothing unusual. I have seen nothing at all in these to indicate that they undergo any transition to a trypaniform phase. The same observation applies equally, I am sorry to have to say, to Halteridium noctuæ, where, in one or two cases of very strong infection, I have found free individuals, of varying size, to be quite numerous.

As I pointed out in my note, the possession by an intracellular parasite of nuclear dimorphism, in the sense in which I have used this term, is very significant and important evidence in favour of a flagellate affinity or connection of the parasite exhibiting this feature. Indeed, on à priori grounds, the undeniable occurrence of this distinctive character in Halteridium is, even regarded by itself, a very weighty argument in support of Schaudinn's view of the ontogenetic connection of this intra-cellular form with a trypanosome. When, in addition, the other evidential points to which I alluded were taken into account, such as the occurrence, now and then, of individuals attempting (as I consider) to develop a flagellum, and the occurrence of very small trypanosomes at the same time, which were no larger than the full-grown Halteridia, the most reasonable conclusion did appear to be that the two forms of parasite were indeed connected.

I admit, nevertheless, that I am now doubtful of such an actual connection, especially since I have been working at Rovigno. I am more inclined to think that an intra-cellular parasite may exhibit nuclear dimorphism, in certain conditions or phases as a result of a close phylogenetic relationship with a parasitic flagellate (say a trypanosome), without necessarily being any longer ontogenetically connected with one. Put into other words, this is to say that a parasite, such as Halteridium, which shows this feature, is probably derived from a trypanosome which has become adapted entirely to a resting, intra-cellular condition, and has coincidently lost, more or less completely, the ability to develop an active trypaniform phase.

Berliner, in a recent paper entitled "Flagellaten-Studien" (1), has incidentally corroborated my account of the occurrence of nuclear dimorphism in Halteridium by describing it in the case of H. noctuæ, i.e. in the very parasite in which Schaudinn first maintained it was present. Berliner's figures are very striking and interesting. His preparations were stained with iron-hæmatoxylin, and another most important point brought out by this method of staining is the close correspondence between the structure of the (chief) nucleus in the Halteridium and that of the trophonucleus of a trypanosome. I need not dwell upon this point here, as Professor Minchin and myself have already referred to it in our paper (13), showing the essential difference which exists, on the other hand, between the nuclear structure of a hæmogregarine and of a trypanosome; and we shall have more to say about it in our own account of the parasites of Athene noctua.

This fact furnishes, however, strong additional evidence in support of the (modified) view of a close relationship between Halteridium and the hæmoflagellates, which I am inclined to prefer. On this view the gradual "Rückbildung" of the kinetonucleus—which is associated principally, of course, with the locomotor activities—can be readily understood, and is, indeed, to be expected. It accounts, further, for the com-

paratively small size of the kinetonuclear element, as well as for the fact that it is not always distinguishable as a separate organella, differentiated from the main nucleus. On the other hand, such a phylogenetic connection of Halteridium with a trypanosome would also render it quite possible that, in certain cases, such as the incidence of an unusual stimulus or under some other special circumstances, the parasites might attempt to pass into—to revert to, as it were—a trypaniform condition. Thus would be explained the peculiar form of individual I have above described, which appears to have developed a flagellar thread.

This view agrees in substance, it will be seen, with Hartmann's ideas (5) of the Hæmosporidia as a whole, which he has united with the hæmoflagellates in one group—the Binucleata—the common character being the possession of a binuclear condition, i.e. of nuclear dimorphism. So far as the hæmogregarines are concerned I do not think they show any evidence at all of this feature (cf. Minchin and Woodcock, l.c.), and therefore consider that these forms, at any rate, should be kept separate.[1] With regard to the malarial parasites (e.g. Plasmodium and Proteosoma), Hartmann considers that these show indications of nuclear dimorphism; apparently, however, the kinetonuclear element is in a more "rückgebildet" condition than is the case in Halteridium. Hartmann thinks, further, that these forms show other evidences of a hæmoflagellate ancestry, such as the presence of a delicate, narrow, undulating membrane, with flagellar border in the microgametes. This opinion was maintained also by Schaudinn in the case of the microgametes of Halteridium.

Not having personally studied the finer structure of the malarial parasites, I cannot say much about Hartmann's opinion. If the above view is correct, as I consider it to be,

[1] In a later paper on this subject, which I have seen just as my MS. is about to go to the press, Hartmann and Jollos ('Arch. Protistenk.,' xix, p. 81, 1910) have apparently come to the same conclusion, and remove the hæmogregarines from the Binucleata.

in the case of Halteridium, there is nothing inherently improbable in supposing that it holds good for the malarial parasites as well; this was, it will be remembered, Schaudinn's idea also. The first essential point, however, is to show that these parasites possess a nucleus (trophonucleus) of the true hæmoflagellate type (such as is shown by the trypanosomes and Halteridium), as revealed by a stain like iron-hæmatoxylin.

As regards the finer structural details of the microgametes of Halteridium, I have been unable to assure myself of the presence of an undulating membrane and flagellar border. I have examined both faintly stained and intensely stained individuals, which, for all I know to the contrary, were as fully developed and mature as if they had been taken from the stomach of the insect; I have studied them with the best objectives and with the best possible illumination. I think the photos reproduced give very accurate representations of these delicate and minute organisms; and neither my friend, Dr. Reid, who has most kindly taken these photos for me, nor I myself, can make out such a structure. It may be there or it may not; I must leave the point unsettled.

Certain of the microgametes in the photos show clearly the centrosomic granule at one end. The opposite end is finely tapering, and comparable to a cytoplasmic tail; as Schaudinn pointed out, it does not appear to be of flagellar nature. The end possessing the centrosomic granule is to regarded as the anterior end; it is by this end that the microgamete penetrates the female element, as can be distinctly seen in fig. J.

As I mentioned in a former section, I examined particularly cultures inoculated with blood containing these ripe gametes, with a view to finding stages in the development of the oökinetes. Somewhat to my surprise, I could find no indications of any developmental changes in the halteridia in the cultures. I saw no oökinete-like phases, and, indeed, only one or two halteridia which had become liberated from the corpuscles, and these appeared to be degenerating and dying.

6. Note on Leucocytozoon fringillinarum, n. sp.

Habitat.—There has been considerable discussion with regard to the exact nature of the host-cell in which these Avian leucocytozoa are parasitic, some authorities stating that it is a leucocyte, while others regard it as an erythroblast, or else an altered red cell. I have been able to assure myself that in the case of this species the host-cell is undoubtedly a uninucleate leucocyte, and not an immature red cell or erythroblast.[1] After once carefully comparing them there is little difficulty in distinguishing between these two types of cell. Examples of immature red cells are seen in figs. 22 and 57, and of uninfected uninucleate leucocytes of about the same size, or a little larger, in figs. 23 and 58. The nucleus of the leucocyte is relatively larger than that of the other type of cell, occupying, indeed, most of the body; moreover, it is nearly always eccentric in position, with the result that the cytoplasm lies chiefly on one side, whereas the nucleus of the erythroblast is central. The appearance of the two nuclei is also different. The latter contains many small chromatic masses; that of the leucocyte, on the other hand, appears to have a few large masses, which by the Romanowsky method of staining do not stand out so sharply from the general nuclear substance as in the other case. Further, the cytoplasm of the leucocyte is always distinctly paler than that of the other kind of cell.

From the immature red cell all transitional stages occur to the ordinary full-sized red blood-corpuscle; but I have seen no connection whatever between such cells and the others—the uninucleate leucocytes—which are entirely distinct. Moreover, in no case have I found the parasites occurring in the former type of cell, but always only in the leucocytes. Wenyon, in his account of L. numidæ (37), figures uninfected cells belonging to this type of immature red cell, above

[1] From the observations which I have so far been able to make upon L. ziemanni, in the little owl, I am strongly inclined to think that the same is true for this parasite also.

described. He also figures a young Leucocytozoon in a cell which obviously corresponds to the uninucleate leucocytes (cf. his fig. 4 with my figs. 24 and 60). But he does not figure the true type of host-cell (uninfected) at all; this, I gather, he considers to be an immature red cell, such as he figures. I have no hesitation in saying—what, indeed, is apparent from my figures—that the uninucleate leucocytes (fig. 23) are the host-cells, and not immature red cells or erythroblasts (fig. 22).

Effects on the Host-cell.—The young Leucocytozoon always penetrates the leucocyte on the side where there is most cytoplasm. It never becomes actually intranuclear, but it often has a curious position in relation to the nucleus during its early growing phases, appearing to be lodged in a deep depression or pit in the side of the nucleus (fig. 62). At times the parasite is almost entirely enclosed by the nucleus (fig. 19). This result is probably due partly to a tendency of the Leucocytozoon to push or sink further inwards, and partly to the growing out or extension of the nucleus, which undergoes a certain amount of hypertrophy, in the form of a wide crescentic or semi-circular mass, at the sides of the parasite. Coincidently, the nucleus undergoes an alteration in character, losing all indications of large, separate chromatic masses, and taking up the stain quite uniformly. As the parasite grows and expands, the free ends of the semi-circular nucleus are pushed outwards, and no longer enclose the Leucocytozoon. When the latter is full grown the nucleus of the containing host-cell is seen as a thick, curved mass at one side (figs. 20, 21, 25, and 26).

In my preparations all the leucocytozoa are intra-cellular. I have never observed more than one parasite in one host-cell.

My observations, as also those of Wenyon (l. c.), of young and intermediate-sized gametocytes, intra-cellular in habitat, and manifestly growing into the adult individuals in a similar situation, do not support in the least Schaudinn's view with regard to the origin of the adult gametocytes.

Schaudinn considered that these were simply the resting-phases of large, sexual trypanosomes, which had come into relation, in a peculiar manner, with the leucocytes, causing the host-cell to become greatly extended and altered in form. I agree with Wenyon that this view cannot be sustained.

Structure of Gametocytes.—In stained preparations the parasites occur in two well-marked and distinct forms, which represent without doubt male and female gametocytes, since they agree very well with these types in other leucocytozoa. The parasites occur in all sizes, from very young forms up to what are probably fully grown, mature individuals (figs. 19, 20, 24, and 25). Even in fairly young individuals the male or female character can be often recognised (figs. 19 and 24). The diameter of a rounded individual averages about $8\frac{1}{2}$ to 9 μ; the ovoid parasite of fig. 26 is 11 μ by $6\frac{1}{2}$ μ. Female forms appear to attain a slightly larger size than male forms.

Comparing a male gametocyte with an individual of female sex, the cytoplasm of the former stains much paler than that of the latter, and appears to be more homogeneous in structure. The cytoplasm of a female individual is distinctly granular. The nucleus of a male form is large and somewhat diffuse; it appears to contain a number of small chromatin granules (probably really chromatin "dust," which stain pinkish. The female nucleus is small, compact and dense; its chromatin grains stain darker and more intensely than in the other case. Both in the male, as well as in the female form, a definite small chromatic body is sometimes found outside, but close to the chief nucleus (figs. 20, 25, and 26); it has also been seen in small parasites (figs. 19 and 60). This small body corresponds to that associated with the nucleus of L. ziemanni, where it was first described by Schaudinn. As I hope to have something to say subsequently, in conjunction with Professor Minchin, upon the nuclear structure of the latter parasite, I will not discuss this point at present, especially as my material is limited and all stained by the Romanowsky method.

One feature about this new Leucocytozoon is of great interest and importance, the fact, namely, that in no instance observed is the cytoplasm of the host-cell extended in the form of a spindle at both sides. Even where the body of the parasite is oval in shape, and more comparable in form to the deeply stained portion of the body in other leucocytozoa, there is no sign of any extension of the protoplasm of the leucocyte. If in the case of other species, e. g. L. ziemanni, L. numidæ, this great drawing out of the ends of the host-cell is due merely to the parasitic influence of the Leucocytozoon, why does the parasite not produce the same effect here? I certainly think it is quite as probable that, in those cases where the spindle-like appearance is shown, there is some more material cause for this constant shape, and that there is really a prolongation of the body of the parasite,[1] in the nature, perhaps, of a faintly staining ectoplasmic layer, for some distance at the two sides, to which is chiefly due this extension of the cytoplasm of the host-cell. Upon this point, also, I shall be able to say more when I have studied the preparations of L. ziemanni. If this is the correct explanation, it is evident that the Leucocytozoon of the chaffinch has lost its ectoplasmic layer, at least so far as can be made out. This development would indicate a closer adaptation to the intracellular condition, which is also seen, perhaps, in the rounded form of the parasite, the other species known being much more fusiform.

I propose the name L. fringillinarum for this new species of Leucocytozoon from the chaffinch; the parasite found by Stevenson in the greenfinch probably also belongs to this same species, since, so far as I can ascertain from the preparation kindly given me by Stevenson, it also has the rounded form and does not cause the host-cell to become spindle-shaped.

Of the many species of Leucocytozoon now known, only two or three, so far as I am aware, have been described as having the rounded form, and with the host-cell lacking the

spindle-like prolongations. The descriptions of these forms are to be found in a series of notes by Mathis and Léger (**10A–10D**). I wish to point out that as regards one at any rate, and possibly more than one, of their parasites, the authors, in describing the gametocytes (and their host-cells) as rounded, appear to have been dealing simply with individuals which had begun the active process of rounding themselves off preparatory to rupturing the host-cell and becoming liberated as ripe gametocytes. Now, in preparations of the fusiform species (L. ziemanni and others), which show gametocytes caught in this act, it is generally impossible to recognise any longer the typical fusiform shape, the cytoplasm of the host-cell having been quickly disorganised.

In the case of Mathis and Léger's forms L. caulleryi (a rounded form) and L. sabrazesi (spindle-like), both from the same host, namely a fowl (Tonkin), I feel sure that the latter parasite is the typical intra-cellular form of the former. Thanks to the authors' kindness in sending some of their preparations of these parasites to the Lister Institute, I have been able to compare them. On a slide containing L. caulleryi all the individuals found are quite rounded-off, and, moreover, there is no sign of the host-cell in connection with them, i.e. the latter has been ruptured and disorganised, and the parasites are seen as ripe, free gametocytes. A slide containing L. sabrazesi, on the other hand, shows the parasites still within their host-cell, the latter having the usual spindle-like prolongations. Mathis and Léger themselves say, in their note on L. caulleryi (**10A**), that only exceptionally did they see the nucleus of the host-cell—evidence that the latter had been ruptured and disorganised. Hence I myself have no doubt, especially when the fact of these two parasites being found in the same host is considered, that L. sabrazesi is only a synonym for L. caulleryi, and that this species (L. caulleryi) belongs really to the fusiform group.

On the other hand, in the case of the species I have described, L. fringillinarum, there is no doubt that it is

quite distinct from the fusiform group, since in all stages—from young forms right up to large gametocytes—the parasite and its host-cell retain the rounded form. Apparently Mathis and Léger's form, L. marchouxi, from Turtur humilis (**10**c), also agrees with this type, for in this case the authors find the host-cell intact, the whole appearance of parasite and leucocyte being, so far as can be judged from the account, similar to that of L. fringillinarum.

The Lister Institute,
April, 1910.

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EXPLANATION OF PLATES 27—31,

Illustrating Dr. H. M. Woodcock's paper "I. On certain Parasites of the Chaffinch (Fringilla cœlebs) and the Redpoll (Linota rufescens)."

[All the drawings on Pls. 1—4 are drawn to a uniform magnification of 2000 diameters. For several of the coloured figures on Pl. 1 and for two or three of the drawings on each of the other plates I am indebted to Miss Rhodes, who has kindly done them for me.]

Plates 27 and 28. With the exception of figs. 7–13, 22, 23, 57, and 58, all the figures relate to the parasites as found in the birds.

PLATE 27.

Figs. 1–6.—Trypanosoma fringillinarum, n. sp.

Fig. 1.—Adult, ordinary individual from the blood of a chaffinch inoculated from a redpoll-culture.

Fig. 2.—Ditto, from the bone-marrow of a naturally infected chaffinch.

Figs. 3 and 4.—Slightly smaller forms; 3, from the blood of a redpoll; 4, from the bone-marrow of a chaffinch.

Figs. 5 and 6.—Small forms of the fusiform type (case B), from the bone-marrow of a chaffinch.

Figs. 7–13.—Cultural forms of the trypanosome; 7–12 from chaffinch cultures; 13 from a redpoll one.

Figs. 7–9, and 13.—Trypanomonad forms (6 and 7 days).

Fig. 10.—Early trypaniform type (40 hours).

Figs. 11 and 12.—Examples of equal and unequal binary fission.

Figs. 14–18.—Halteridium fringillæ (Labbé).

Fig. 14.—Female individual.

Figs. 15 and 17.—"Indifferent" individuals.

Fig. 16.—Special form, free in the blood-plasma, with chromatic

line. (Unfortunately the terminal granule has not come out in the plate.)

Fig. 18.—Very young form.

Figs. 19–21, 24–26, Leucocytozoon fringillinarum, n. sp.

Figs. 19 and 24.—Young gametocytes, female and male.

Figs. 20 and 26.—Large female gametocytes.

Figs. 21 and 25.—Large male gametocytes.

Fig. 22.—Immature red blood-corpuscle.

Fig. 23—Leucocyte (uninfected).

PLATE 28.

Figs. 27–56.—T. fringillinarum.

Figs. 27–33.—Ordinary definitive forms of the parasite of varying size; 27 from a chaffinch inoculated with redpoll culture, 28–31 from naturally infected chaffinch, 32 and 33 from naturally infected redpoll.

Figs. 34–38.—Large, massive forms, from a redpoll.

Fig. 39.—? Transitional form, intermediate between ordinary type and that last mentioned, from a redpoll.

Figs. 40–45.—Series of fusiform parasites from very small to a moderate size, from a chaffinch (Case B).

Figs. 46–54.—Small forms from a chaffinch (Case A), fusiform or broad and stumpy; 48 and 54 show indications of division. Many of the individuals in both these series show the granular chain or line.

Figs. 55 and 56.—Remarkably slender individuals (? young, definitive forms).

Figs. 57 and 58.—Immature red blood-cell or erythroblast and uninfected leucocyte, respectively.

Figs. 59–62.—Leucocytozoon fringillinarum.

Fig. 59.—Male individual.

Fig. 61.—Female individual.

Figs. 60 and 62.—Young forms, probably female individuals.

Figs. 63–70.—Halteridium fringillæ.

Figs. 63, 65, and 66.—Medium-sized to large female forms.

Fig. 64.—"Indifferent" individual.

Figs. 67–69.—Small or intermediate-sized individuals.

Fig. 70 *a* and *b*.—Male gametes.

PLATE 29.

Figs. 71–111.—Cultural forms of T. fringillinarum.

[All the figures are from original cultures of 6–8 days, except figs.

74 and 109, which are from a second sub-culture of 26 days, specially for comparison.]

[(C) indicates chaffinch culture; (R) redpoll-culture.]

Figs. 71–86.—The ordinary trypanomonad type, showing variations in size and in degree of development of the membrane.

Figs. 71–76, 83–86 (C); figs. 77–82 (R).

Figs. 87 and 88.—Individuals in which the kinetonucleus is a trifle on the aflagellar side of the trophonucleus; in fig. 88 division is just being inaugurated. Both (C).

Figs. 89–95.—Stages in equal binary fission. All (C) except fig. 83, which is (R).

Fig. 96.—Division-form of sub-equal character, giving rise to individuals of the accentuated trypanomonad kind.

Fig. 97.—Accentuated trypanomonad individual (C).

Figs. 98–104.—Various stages in the unequal division of the accentuated trypanomonad individuals. Figs. 100 and 103 are (R), the rest are (C).

Figs. 105–111.—Illustrative of the two kinds of individual which result from unequal fission. Figs. 105, 107 (upper half), 108–110, accentuated trypanomonad forms, often more or less club-shaped, with nuclei far back and well-developed membrane; Figs. 107 (lower half), 106 and 111 A and B, fusiform individuals, with only slightly developed membrane; note the comparatively short flagellum. Figs. 106 and 108 (R), rest (C).

PLATE 30.

Figs. 112–131, 133–163.—Cultural forms of T. fringillinarum (contd.)

Figs. 112–114.—Pear-shaped forms, probably derived from the smaller halves of unequal divisions, which have not become fusiform. Figs. 112 and 114 (C), 113 (R).

Figs. 115 and 118.—Small and large individuals of the accentuated trypanomonad kind, passing into the ovoid or rounded condition. Both (C).

Figs. 116, 117, and 119.—Medium-sized rounded forms. (All C).

Figs. 120–125.—Individuals from the (C) culture which showed a pronounced tendency to develop large massive forms. Many of them are undergoing division.

Figs. 126 and 127.—Early trypaniform individuals. (C) forty hours.

Fig. 128.—? Transition form from trypanomonad to trypaniform type. (C) 6 days.

Fig. 129.—Small trypaniform individual. (C) 6 days.

Figs. 130 and 131.—Greatly elongated trypaniform individuals. (C) 6 days.

Fig. 132.—Trypaniform phase of a trypanosome of Athene noctua from the stomach of Culex pipiens.

Figs. 133, 134, and 136.—Rounded forms still possessing a flagellum, but lacking any signs of an undulating membrane. In the two first a large vacuole is present. Fig. 133 (C), figs. 134 and 136 (R).

Figs. 135 and 137.—Rounded forms without a flagellum (R).

Figs. 138 and 139.—Small rounded forms in a dying condition; the two nuclei are gradually disappearing (R).

Figs. 140–146.—"Pseudo-herpetomonad" forms, illustrating various degrees in the loss of the membrane and attached part of the flagellum. Figs. 140, 145, and 146 (C), 141–144 (R).

Fig. 147.—Herpetomonad form (C).

Figs. 148 and 149.—Pear-shaped forms, with little or no attached part to the flagellum (R).

Figs. 150–163.—All these forms are from a (R) culture of 19 days.

Figs. 150–153.—Large, altered, unhealthy parasites, with a development of granular substance in the region of the base of the flagellum.

Fig. 154.—Parasite showing two large vacuoles.

Fig. 155.—Individual with a kinetonucleus, but no trophonucleus.

Fig. 156.—Individual with one kinetonucleus and two trophonuclei.

Fig. 157.—Dividing parasite, showing how the unequal distribution of the nuclei, as found in the two last forms, is brought about.

Fig. 158.—Parasite with three trophonuclei for one kinetonucleus.

Fig. 159.—Showing the splitting off of an individual with a flagellum, but with no nuclear substance at all.

Fig. 160.—A free, active individual, with no definite nucleus of either kind, but with scattered granules.

Fig. 161.—Showing the splitting-off of a portion of the cytoplasm containing only a few granules.

Figs. 162 and 163.—Forms showing irregular multiplication of the different organellæ.

PLATE 31.

[The micro-photographs on this plate were all taken for me by my friend Dr. D. J. Reid, to whom I wish here to express my deep sense of his kindness and to offer my sincere thanks. It is as well to point out, perhaps, that the more deeply stained parts have come out, in most cases, relatively far too dark.

The magnifications are as follows (approximately): Figs. A–D 1630, fig. E 620, fig. F 500, fig. G 550, figs. H and J 1630, fig. K 1840, fig. L. 1220.]

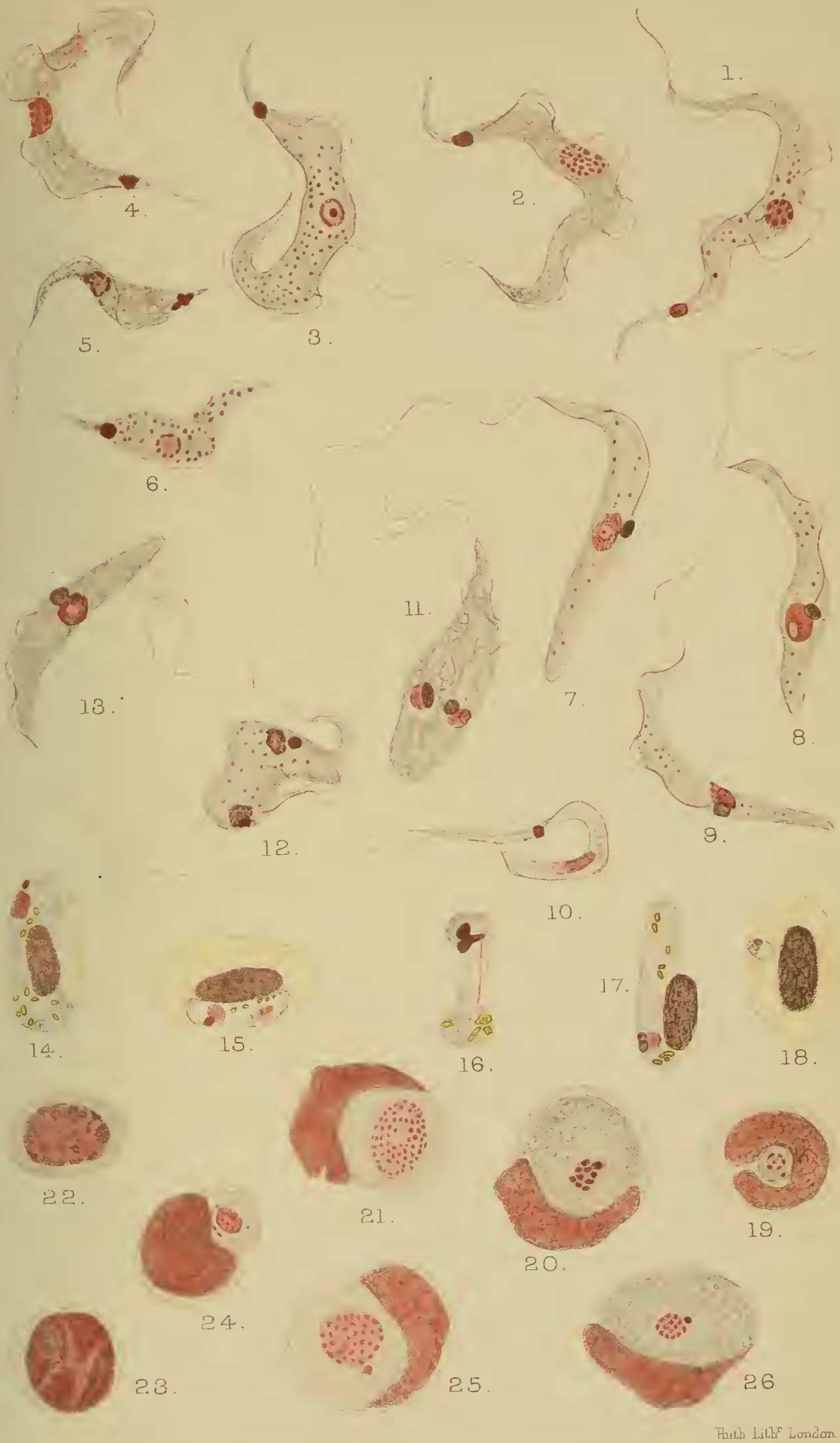
Figs. A–D.—Trypanosoma fringillinarum, as found in the birds. For description of these figures see under figs. 2, 3, 28, and 54, which are of the same individuals respectively. [In the reproduction the whole length of the delicate aflagellar prolongation, which is visible in the actual photos, cannot be made out. Unfortunately there are two small pieces of débris lying on the parasite of fig. B, which are, of course, reproduced. One lies about one third of the distance from the kinetonucleus to the trophonucleus; the other on the fold of the membrane opposite to the nucleus. In the drawn figure (fig. 3) these particles are omitted.]

Figs. E–G.—Agglomeration clusters of various sizes of T. fringillinarum in cultures. [The parasites of the first two clusters are not so nicely stained, unfortunately, as those of the third, but they show the manner of formation of the cluster.]

Fig. H.—Halteridium fringillæ; female individual showing nuclear dimorphism (the same is drawn in fig. 14).

Fig. J.—Fertilisation of a macrogamete by a microgamete. Note that the latter is penetrating by the end which has the centrosomic granule.

Figs. K and L.—Microgametes.



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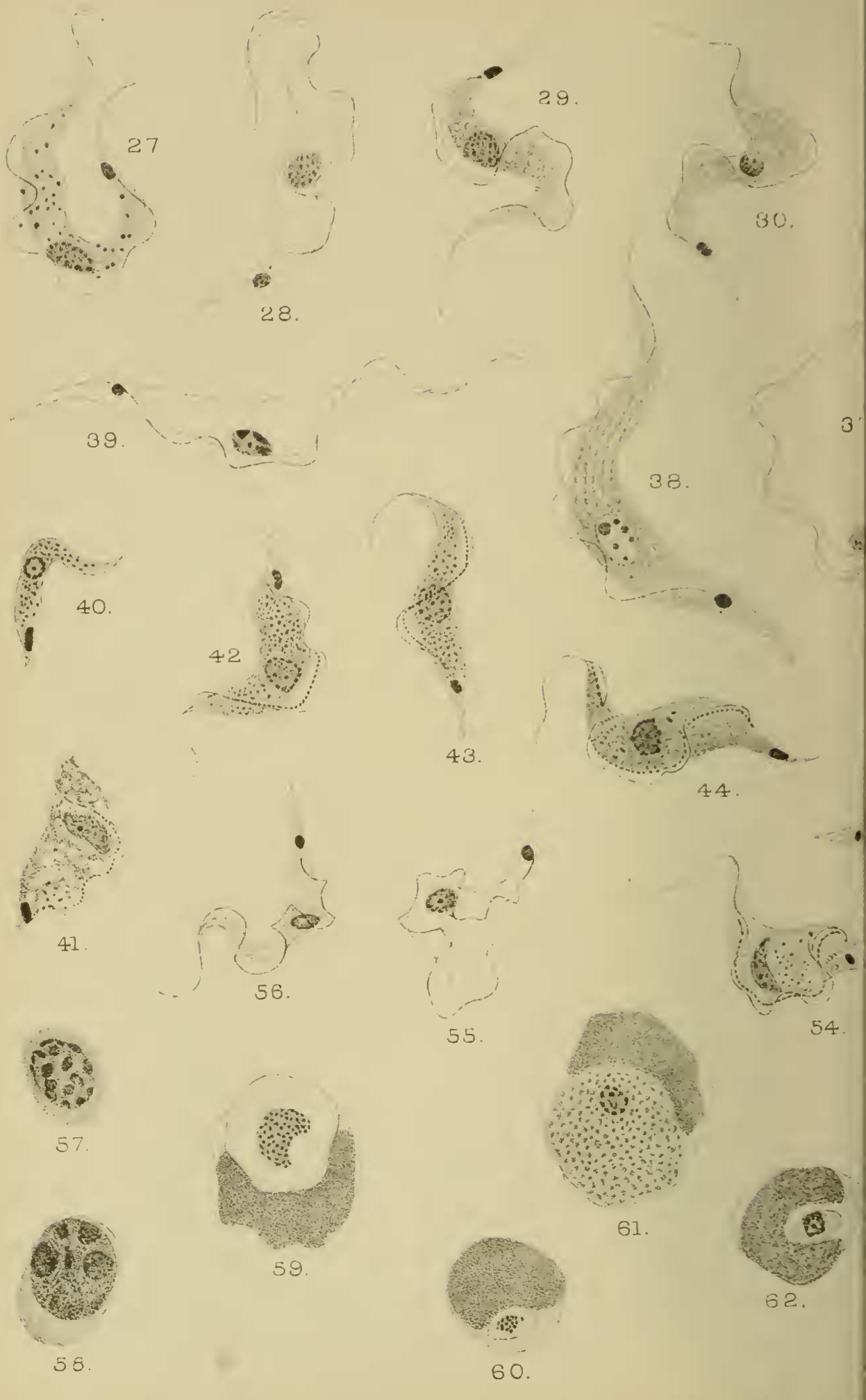
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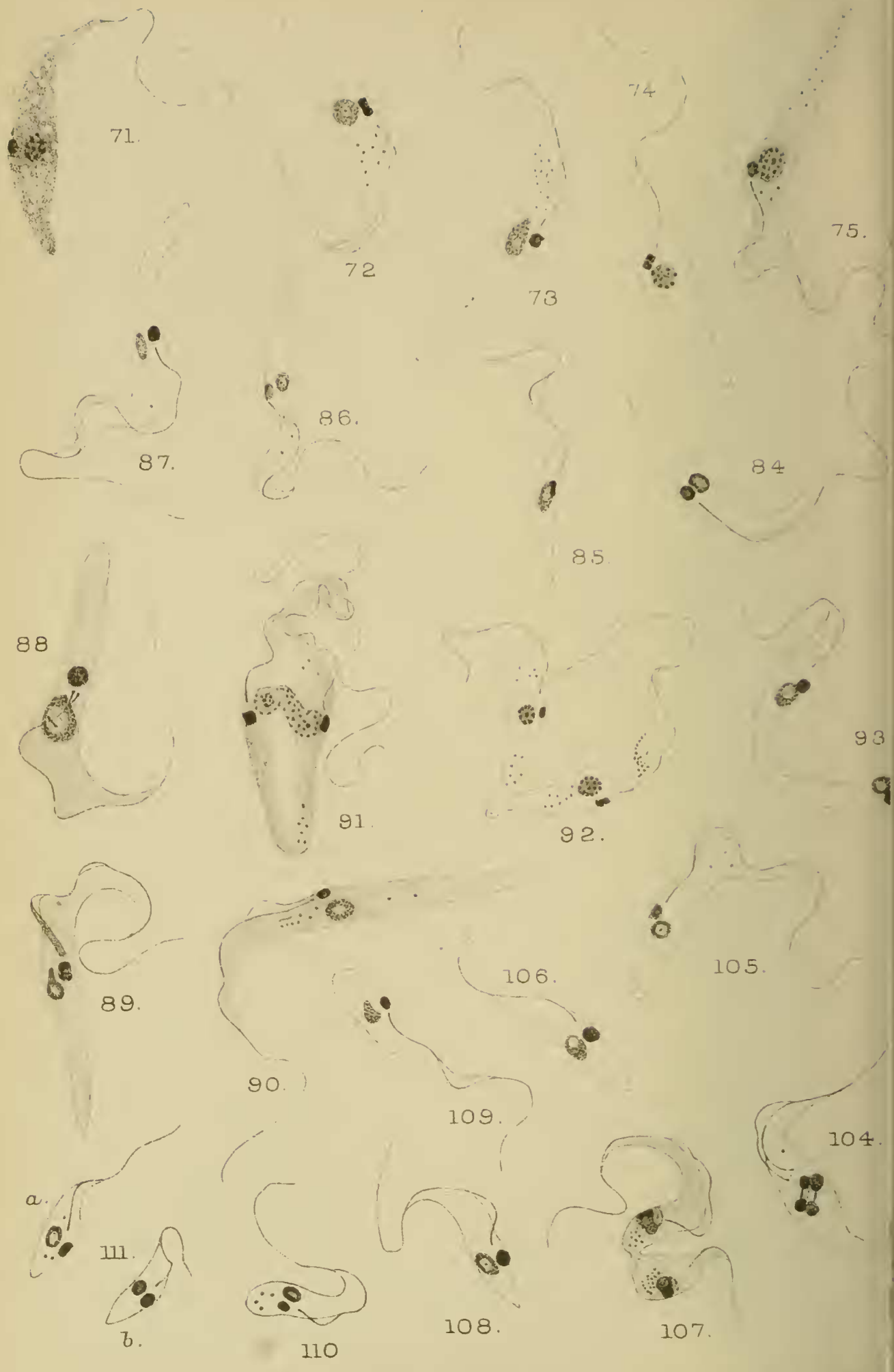
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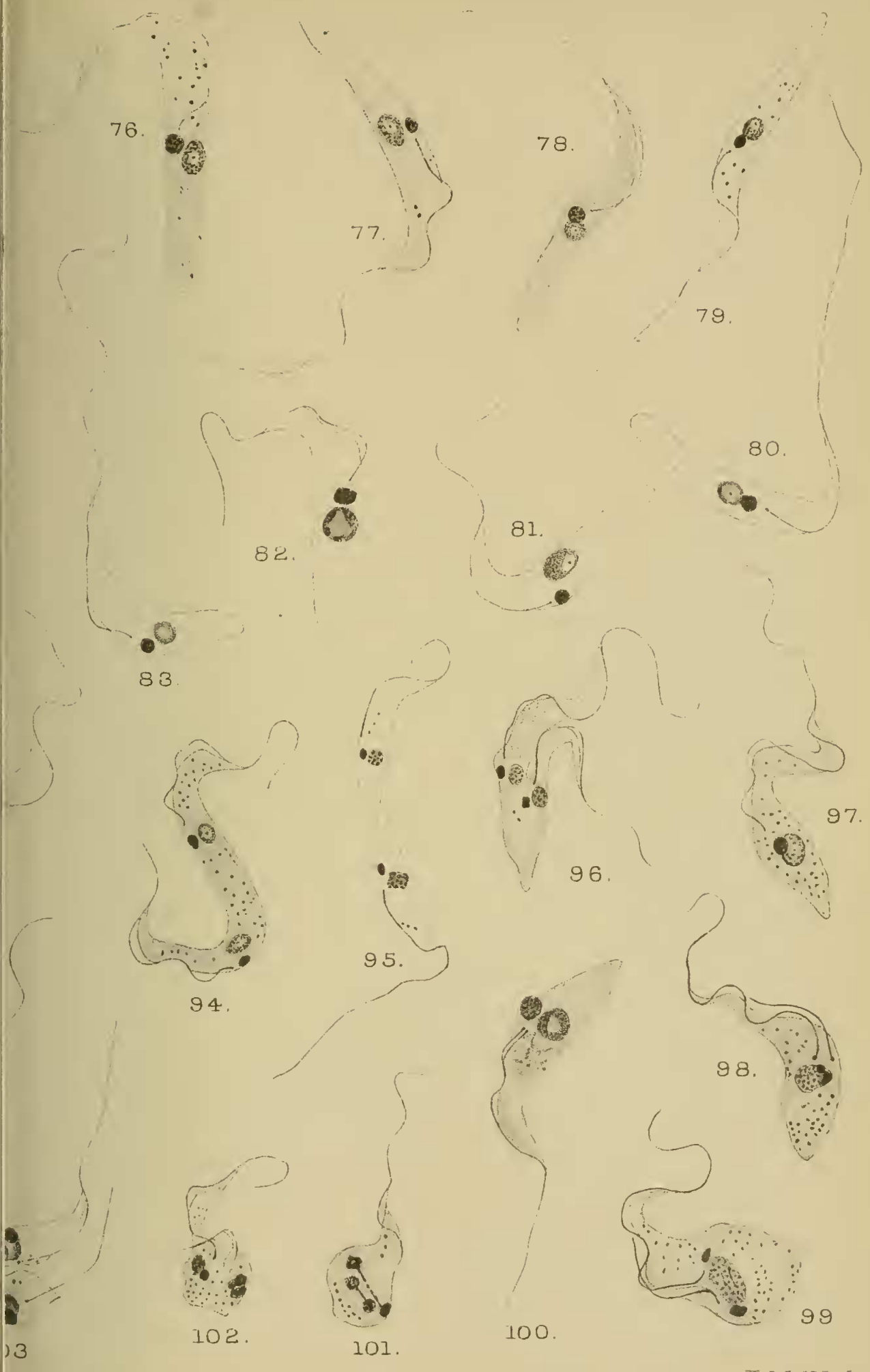

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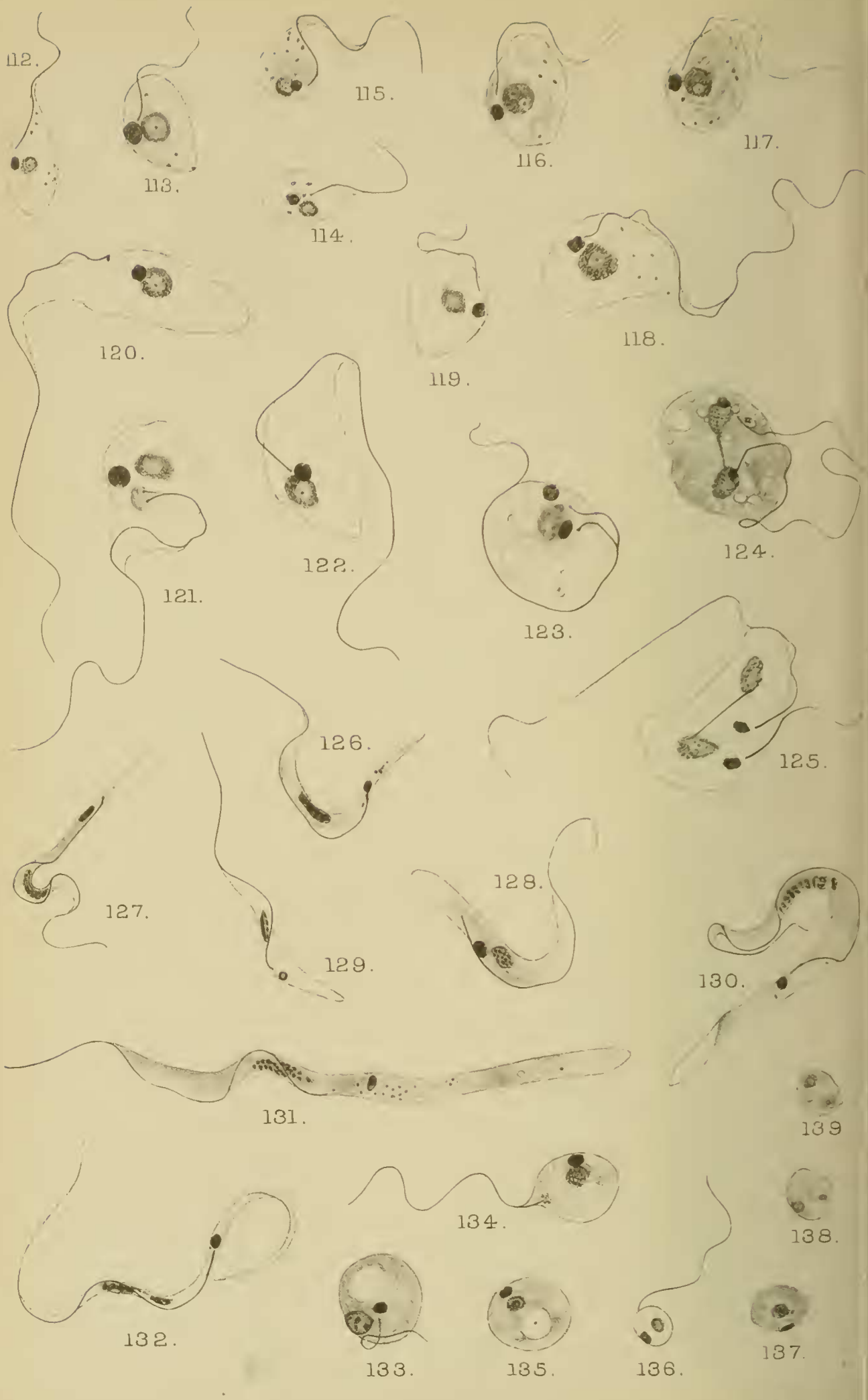
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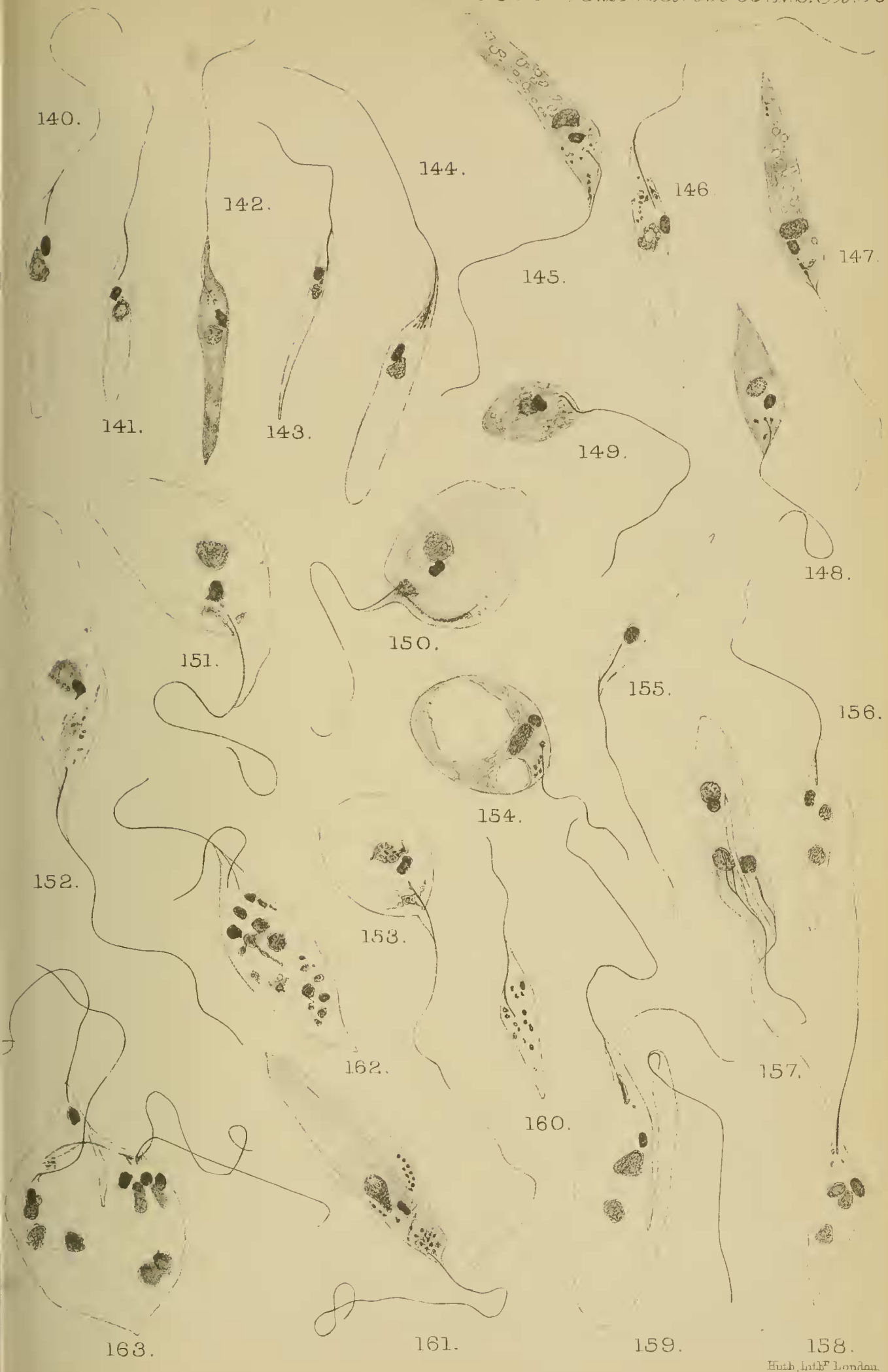

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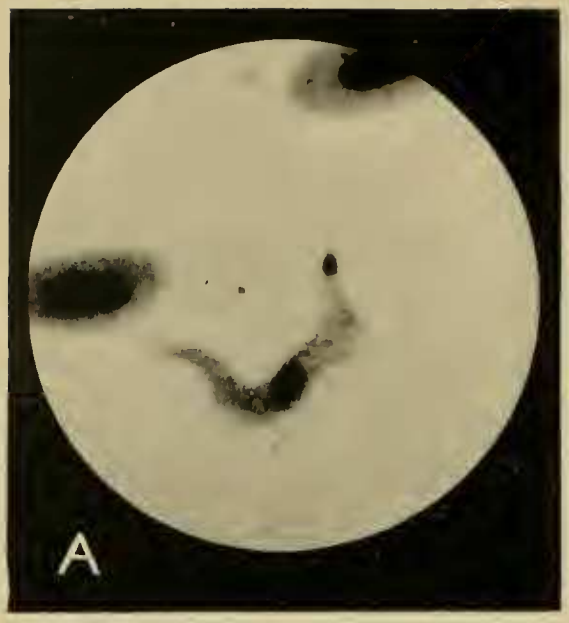
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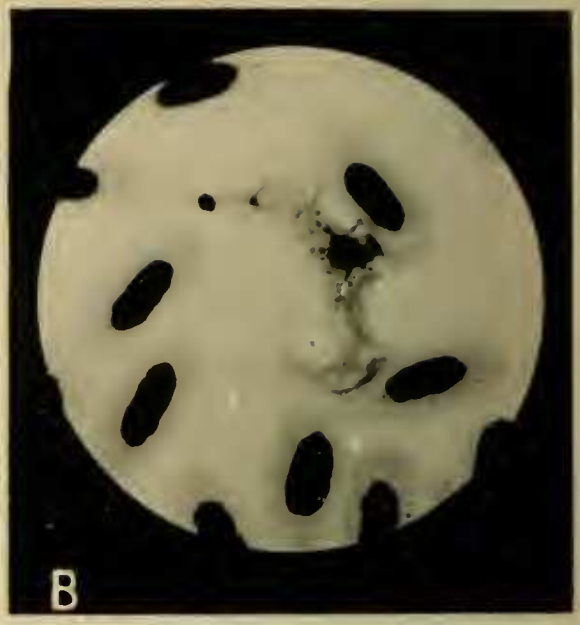
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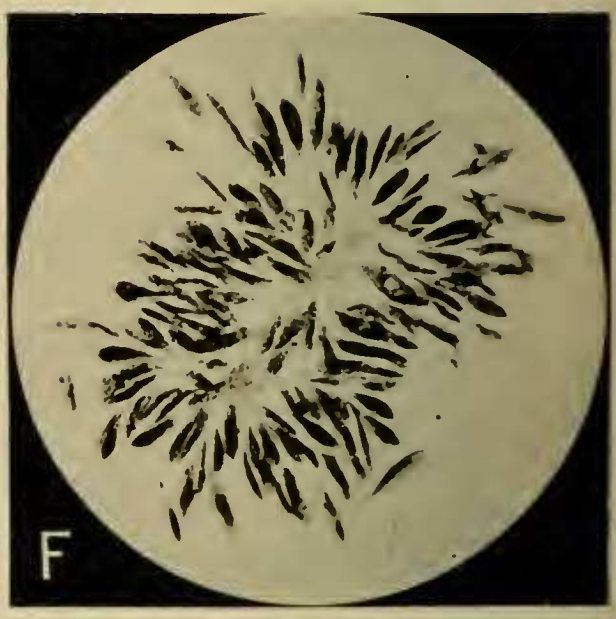
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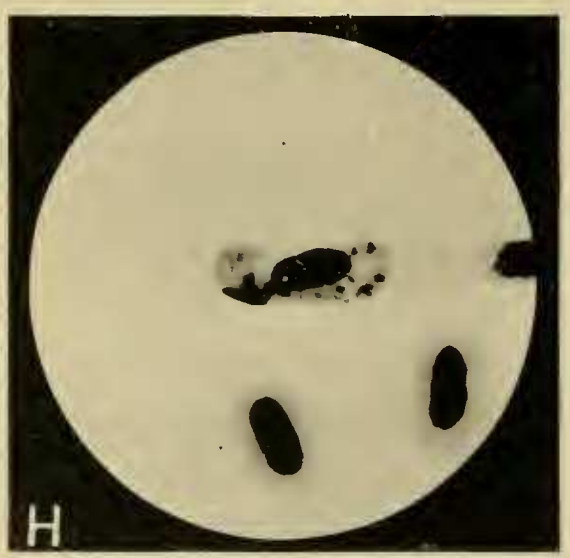
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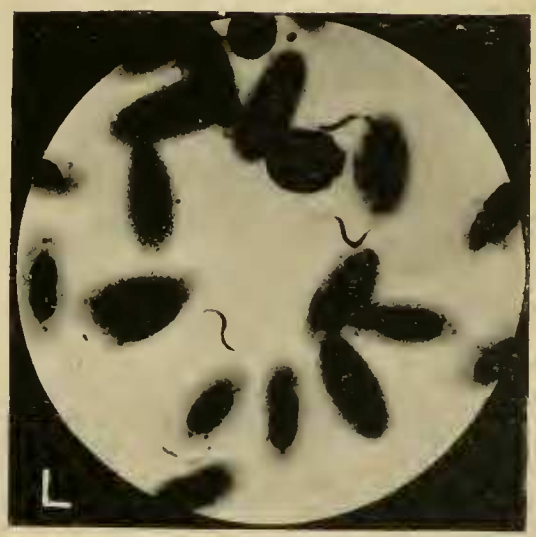

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